

10 September 1981

# No nuclear battlefield in Europe

The Reagan Administration has not long to begin negotiations with the Soviet Union on arms control. Unwelcome though it may be, to a government more jealous of its sovereign rights than most of its predecessors, to be told that its choice may be circumscribed in any way, the plain truth is that further delay is likely to jeopardize the cohesion of the alliances on which the United States has laboured for the past thirty years. For the pressure to which the Administration is now exposed is neither technical nor generated by the Soviet Union but, rather, is the most awkward pressure of all — of the nature of things.

President Reagan's deadline must lie ahead — prudently far ahead — of the party meeting next April of the West German Social Democratic Party, of which Chancellor Helmut Schmidt is a member. The party's informal meeting last month showed clearly that the chancellor's previously resolute support for Western strategic doctrines entails substantial political risks. There is at least a chance that the party, frustrated by the continuing stalemate on arms control negotiations, will come out against the siting of United States nuclear weapons in West Germany. If, by disowning what Herr Schmidt has stood for all these years, the party forces the collapse of the uneasy coalition over which he presides and thus a general election in West Germany centred on the issue of the defence of Western Europe, the fat will truly be in the fire. The Reagan Administration may also be given pause by the impending election of a deputy leader of the British Labour Party, for there is a chance that Mr Denis Healey, once a Minister of Defence and a solid NATO man, will be turned out by a colleague committed to some form of unilateralism. Even though the consequences of such a development cannot be foreseen, Washington will be given the sense that the rot has set in in Europe.

Why has all this happened so quickly? The neutron bomb is less urgent a trigger of disaffection in Western Europe than the earlier decisions (agreed within NATO) that Pershing and cruise missiles should be based in Western Europe. To the extent that it is within the range of these devices to be used in attacks on the Soviet Union, there is a prospect that by the end of 1982 (when the first missiles are due to be installed), parts of Western Europe will be regarded as strategic bases. But have not Montana and Wyoming been vulnerable for the same reasons all these years? To many Europeans, the comparison is far from comforting. For now it is possible for Europeans to fear that the West might fight a nuclear war without involving the continental United States — an impression, perhaps a misapprehension, that has if anything been reinforced by the tactless way in which officers of the still new (or learning) Administration have been talking in the past few months about the feasibility of nuclear war as if it were just another public project such as the synfuels programme.

One obvious consequence has been to encourage the several voluntary organizations in Western Europe which exist to protest against nuclear weapons, nuclear strategy and nuclear war. The way in which this abreaction works can be inferred from Mr Edward Thompson's eloquent speech to the British Association last week (see page 93). Mr Thompson is a distinguished historian who is one of the leaders of European Nuclear Disarmament (called "END") and who has recently become even better known because the BBC withdrew an earlier invitation to him to give a public television lecture. Mr Thompson's argument last week was simple. The theory of deterrence, according to Mr Thompson, is not a theory at all, but a *post hoc* justification for the accumulation of weapons by the superpowers; how else can the

theory be used to endorse "every new development in strategy and weaponry"? Mr Thompson made a fair point when he argued that to describe the British nuclear weapons force as "the deterrent" is pejorative. He went on to argue that the theory of deterrence, coupled with the habit of the superpowers of preparing themselves to contend with the worst that their opponents could effect — effective counter-force strategies, for example — served only as a means of fuelling the arms race. So, for intellectuals like himself, the only acceptable course of action is to pretend that the theory of deterrence does not exist, but to attempt to "put European culture back together" without the help of politicians.

Variants of this argument are now everywhere to be heard. In West Germany and several other European countries, a host of voluntary groups, some of them including only specialists, have sprung up to advance versions of Mr Thompson's argument. And, in the past few days, some physicians who are also members of the National Academy of Sciences, and including several people usually thought of as conservative, have put out a statement expressing their concern, even alarm. The Administration can ignore these tendencies only at the peril of the strategic doctrine to which it is committed. First, it must work out afresh the position of the United States on nuclear strategy, and on the strategy of deterrence in particular. Mr Thompson and his West German equivalents apart, there is no reason to suppose that the theory of deterrence is less appropriate now than in the years since the Second World War. The underlying argument is simple — nuclear weapons in the hands of one superpower or the other are rendered unusable by the fear of the limitless damage that would be caused by retaliation. Throughout the past thirty years, governments in the West have been reconciled to the notion that circumstances could arise in Europe where the use of nuclear weapons would be the only effective device — and have calculated that, if there should then be retaliation from the other side, the ultimate consequence would be that the United States guarantee of Western Europe would come into effect, whatever the consequences for the United States.

Why then are so many people in the West now afflicted with doubt on this all-important question? First, loose talk in Washington has created the false impression that Europe is potentially an isolated nuclear battlefield. Second, the temper of the Reagan Administration, with its apparent indifference to European susceptibilities, has led some people to wonder whether the old guarantee still stands as firmly. Third, events in Poland have persuaded some unrealistically to hope that nuclear weapons would go away, so that Europe as a whole might become a more flexible and civilized place. Finally, and properly, there is a growing sense of regret at the huge resources being lavished on the improvement of nuclear arsenals, both in the East and West, when there are so many more constructive uses to be made of economic resources, and when the sheer numbers of warheads deployed lends mistaken credence to the belief that the more weapons there are, the greater the chance that they will be used.

President Reagan's only constructive course, in such circumstances, is to do now what it has been plain ever since Inauguration Day that he must do soon — seriously to take up one of the several opportunities for talking to the Soviet Union on arms control. But what to talk about? The best course would be to acknowledge that the second instalment of the agreement on strategic arms (SALT 2), limited as it is in content and never ratified, had better be replaced by a more comprehensive treaty



on long-range nuclear weapons. In parallel, there is a case for beginning negotiations on nuclear weapons based in Europe recognizing, at the same time, that the technicalities of this problem are formidable. The feasibility of a comprehensive test-ban treaty has receded, but it should not be forgotten that, just a year ago, officials in the then United States Administration were saying that only a month or so was needed to bring the treaty to a conclusion. These are only some of the avenues that might usefully be explored. The need is not to keep Chancellor Helmut Schmidt in office (for he can look after himself, and should be doing so more effectively) but to demonstrate to Western Europe and the rest of the world that the agreement within the West on which the whole theory of deterrence has been based has not, since January, become a dead letter. For President Reagan, the delicate first task is somehow to persuade the United States military, together with a substantial part of the American electorate, that they like others can live with arms control.

## One hope retracted

The news that Professor Efraim Racker has found it necessary to withdraw from publication a paper read at the Cold Spring Harbor Tumor Virus Symposium last May, and has in the process cast doubt on several related papers, is a cruel disappointment. For several years it has been apparent that in the understanding of the causation of cancers of various kinds, the most clamant need has been for a mechanism of some generality that would explain how it comes about that carcinogens of very different kinds (chemicals such as benzpyrene, radiation or just age) can cause malignant diseases that take on a life of their own. Mr Mark Spector's account of how the products of RNA tumour viruses, more often known as retroviruses, might affect the biochemistry of a cell, promised to be just such a mechanism. If, indeed, a succession of steps, chemically similar, amounting to the phosphorylation of successive protein in a hierarchy of protein kinases had been involved in the manifestation of cancer caused by retroviruses, it would have been relatively easy to understand how different carcinogenic agents might affect different steps in the ladder.

So what has gone wrong? That is what people working in the field will now be asking. And those members of Congress who have been wringing their hands for the past few months about the warts on the fair face of science, and who have only grudgingly accepted the assurances offered them by the scientific community, will no doubt be opening yet another inquiry into what seems to be yet another case of inexplicable error. At this stage, it is far too soon to guess what Congress will make of what has been happening at Cornell, but certainly the circumstances do not resemble those of the cases of outright deception that have created such a sense of shock in the past eighteen months. First, the people who have blown the whistle on this occasion include many of the authors of the original and now suspect research. Second, it is reported that some parts of the proposed mechanism of carcinogenesis were accurately described. Third, retraction has been swift and none of the participants seems to have benefited personally, even in the intangible ways that often go with successful innovation in such a spectacular field.

Unfortunately, that cannot be an end to the affair. Self-deception, when published, is also culpable. On this occasion, the conspicuous case of self-deception seems to have been that of Mr Mark Spector, by all accounts a dedicated graduate student given to herculean labours. The question will, however, be raised whether those among his seniors who put their names to his account of his research can emerge unscathed from the mess that has been created. The difficulty is that by convention — not merely a convention of the scientific literature but of scholarship and intellectual intercourse in general — people who put their names to a publication must be able to defend what has been said. Again on the face of things, this seems not to have been the case at Cornell. After the Cold Spring Harbor meeting, Professor Racker seems to have checked his junior colleague's work. Should

he not have been there at the beginning?

What, in these unhappy circumstances, should be done? Internally, as a free-standing university of some repute, Cornell needs to discover for itself what has gone wrong. With a little luck, it may discover that there is something to be salvaged. There is, however, a chance that it may also discover that there is something wrong in the usual relationship between junior and senior investigators (who spend respectively too much and too little time at the bench). The awkward truth about experimental investigations is that no amount of preliminary planning and no amount of eventual statistical analysis (or some other form of assistance) can adequately substitute for having measured something or having observed it. Those whose administrative and other extra-laboratory responsibilities are too pressing to permit the strict observance of this rule should acknowledge the risks that they are running. And those in Congress who have been speculating, during this year's seemingly endless inquiries on the question of falsification, that the penalties of failure have become intolerably great, should now give some thought to the possibility that the rewards of success have also been too great.

## Students vote with feet

In the British system of higher education, this is the most poignant season of the year. For this is when would-be students who have been refused by the universities to which they first applied for entry are matched (by the Universities' Central Council on Admissions) with the vacancies still left within the system. The euphemism for the process is the "clearing". The process is largely mechanical. Students have already declared their interests, and the council's computer can be reckoned on to ensure that the best qualified among such would-be students as wish to spend the rest of their lives in the study of, say, Sanskrit will find their way to the establishments offering instruction in such arcane pursuits. In reality, of course, there is no conspicuous institution within the British educational system that offers to gratify such ambitions. There are, however, many universities that have informed the central computer that they could accommodate so many students in modern languages, the humanities, medicine and the sciences.

Last week, the council put out a statement to the schools warning head teachers that, this year, the "clearing" would find places in higher education for fewer people — perhaps half as many — than it was able to accommodate last year. Would-be scientists will be frequently disappointed, for young people (no doubt by a quick reading of job advertisements in the newspapers) have apparently worked out that technical skills are more marketable than other kinds of skills. The result is that would-be Sanskrit students are even more likely to be satisfied by the clearing, and that more would-be scientists than ever will join the dole queues.

This is a multi-dimensional nonsense. The University Grants Committee, remarking that science and its application is probably in the national interest, as well as the upturn in enthusiasm among young people, has decreed that the numbers of students on science courses should be reduced by a smaller proportion than the numbers of students following other kinds of courses. But there is nevertheless to be a reduction, of the order of five per cent in the next three years. The difficulty is that nobody can at this stage know whether the willingness of universities to educate different kinds of students corresponds with the government's intentions. Some may even have compromised with the wish of their Sanskrit teachers (and the like) to retain their tenured posts. Accordingly, nobody should think of crying scandal if it turns out, when the "clearing" is done with, that the dole queues are more richly endowed with would-be scientists than with other disappointed university students. That, after all, is how the system has been constructed. The tragedy is that in the first year in ten in which there has been a substantial resurgence of interest in learning science, British universities should have found themselves unable to respond.



# Spectacular cancer mechanism doubted

## Cornell retracts reports of kinase cascade

### Boston

The authenticity of one of the year's most exciting biochemical announcements has been cast in doubt. The remarkable biochemical pathway presented at the 20–24 May Cold Spring Harbor Tumor Virus meeting, the "kinase cascade", has slipped back into the ranks of the unconfirmed. This provocative scheme, described to the meeting by Mr Mark Spector, offered a mechanism whereby the products of RNA tumour virus transforming genes initiated a sequence of reactions that helped explain how a normal cell is converted into a tumour cell.

Professor Efraim Racker, of Cornell University, in whose laboratory the work was done, has now sent a carefully worded retraction to *Science* and *Cell* withdrawing his confidence in some of the data from his laboratory's latest work and its recent publication in the pages of those two journals. In his retraction, Professor Racker says that although he believes the major concepts and components of the protein kinase cascade are correct, he doubts the authenticity of some of the data. Professor Racker also hopes to substitute a new paper for one he submitted to the Cold Spring Harbor Symposium, thus preventing further unreliable data from reaching publication.

The retraction Professor Racker is sending to *Science* and *Cell* is problematic because although his group has had difficulty reproducing results pointing to a cascade, such a pathway may turn out to be correct.

The presentation of the "kinase cascade" was the high point of the Cold Spring Harbor meeting (see *Nature* 292, 15; 1981) and has been the centre of discussion in the biochemical community since then. The result was so exciting that it was even placed on the front cover of the Cold Spring Harbor abstract book. But some members of the audience were sceptical of the work from the start. Sceptics believed that the results looked too clean and were shocked at the speed with which it had been completed. Professor Racker was confronted with these suspicions soon after the meeting and further questions arose when other laboratories nationwide were unable to reproduce the results.

Professor Racker says that he went into the laboratory himself six weeks ago to try to reproduce the work. He quickly confirmed that the final step of the cascade (the phosphorylation of a tyrosine residue

on the B-subunit of a membrane ATPase), was correct as first reported (*J. biol. Chem.* 255, 5504; 1980).

He later confirmed another interesting result: the identification of a 6,000-dalton polypeptide which activates some kinases and phenotypically transforms cell lines. He has since sent this tumour growth factor to outside laboratories for verification.

But except for these two pieces of good news for those rooting for the cascade, all attempts at reproducing previously reported results have failed. Professor Racker and his collaborator Dr Volker Vogt (also of Cornell University) who is a co-author of the *Cell* paper, both believe that all experiments reported involving antisera are suspect.

Professor Racker's retraction comes less than a month after the publication of his

latest article. Other than the two confirmed results, the substantiation of all other data will take months to complete. Until then the biochemical community must wait.

*David Dickson adds (from Washington):* Mr Mark Spector is a graduate student at the Department of Biochemistry and Molecular and Cell Biology at Cornell University, where he has a reputation for working eighteen hours a day. Professor Racker said that the paper read at the Cold Spring Harbor symposium had now been withdrawn.

He also said that while some parts of the scheme worked out by Spector had been confirmed, including the role of the 6,000-dalton polypeptide isolated by Spector, "we are finding more and more things that we suspect".

**Michael D. Stein**

## Biochemical cascades and carcinogenesis

The biochemical pathway described in a series of papers published in the past year by Mark Spector and Professor Efraim Racker, with or without others from Cornell University, centres on four new enzymes. The enzymes were said to be linked in a cascade of reactions such that the activation of any one of them had the end result of reducing the efficiency of the cell membrane enzyme that is responsible for pumping sodium out of cells. Only in tumour cells did the cascade appear to be activated and the sodium pump to be inefficient.

The link of this observation with cancer was tenuous but enticing. Otto Warburg, in the 1920s, first showed that tumour cells exhibit an excessive fermentation of glucose to lactic acid, a biochemical pathway that consumes ADP and inorganic phosphate. These are the two products of the hydrolysis of ATP which accompanies the pumping of sodium from the cell and which provides the energy to drive the pump.

An inefficient pump, as described by Spector and Racker for tumour cells, would hydrolyse more ATP than a normal cell in keeping the cellular sodium at acceptable levels. Since that would result in the production of abnormally large amounts of ADP and inorganic phosphate, the observed increase in tumour cells of glucose fermentation was explained.

There was an additional link between cancer and the cascade, forged particularly in the July issue of *Cell*. The infection of many cells by tumour viruses is known to be followed by the appearance of new phosphorylating enzymes in the cell. But exactly what they phosphorylate and with what consequence has remained an enigma. The cascade provided a solution.

In their *Cell* paper, Spector *et al.* presented evidence that the enzyme produced when one particular tumour

virus infected cells was very similar to one of the cascade enzymes. The virus might activate the cascade by the production of increased amounts of one of its members. In other cases it was thought more likely that activation followed phosphorylation of one of the members of the cascade by a virally produced phosphorylating enzyme.

Despite the attraction of this scheme, it came in for sceptical comment from September 1980, with the publication of the evidence that the sodium pump from tumour cells was phosphorylated and rendered inefficient. Doubts centred on the identity of the pumping enzyme and the likelihood of the result, given the artificial — if elegant — system used.

Data published by Spector *et al.* in the 10 May issue of the *Journal of Biological Chemistry*, and in *Cell* (and reviewed in 17 July *Science*) attempted to establish the identity and activity of the cascade enzymes. Doubts about this work centred largely on the speed of progress.

At the Cold Spring Harbor meeting in May, Spector was allowed to give the longest talk of any contributor — his talk was rescheduled to be the last of an evening session so that he could present as much of his data as possible. Even so, much of it had to be passed over at too great a speed for appraisal and it was widely felt that Spector's response to some of the questions was vague. It was also clear that Spector was being cold-shouldered by the majority of senior participants at the meeting. In private, Spector admitted to being hurt by the scepticism but readily agreed that many of his conclusions were tentative and needed substantiation. When it was put to him that even if only 50 per cent was substantiated, the work would still be of great importance, as basic biochemistry even if not related to cancer, Spector said he would be pleased if 20 per cent were confirmed.

**Peter Newmark**



## Weapons control

### Pugwash latest

#### Washington

The thirty-first Pugwash meeting on arms control and nuclear disarmament took place last week at Banff in Alberta, but for the first time in its 24-year history, the Pugwash movement had visa applications rejected for two scientists who had been invited to attend the meeting.

The Canadian government refused permission for two Soviet scientists, Dr Vladimir Paylichenko of the presidium of the Soviet Academy of Sciences, and Dr Vladimir Ustinov, described as a specialist in disarmament and the history of science, to attend the meeting.

No reasons were given by the government for its decision, apart from the statement that it had been made on the grounds of national security.

On the first day of its meeting, the Pugwash council issued a strongly worded protest against the exclusion of the two Soviet scientists, claiming that "the suspicion and distrust which existed in 1957 which our meetings have always tried to dispel still exist, even in a country as open, friendly and generous as Canada".

The statement said that the Pugwash movement had always considered it essential that it should enable people to meet who hold different opinions, come from different backgrounds, and have different experiences. "We regard the exclusion of individuals whose presence we have invited as a breach of this principle against which we most strongly protest."

The council also said that the Canadian government's decision "makes it clear that Pugwash meetings are more urgently needed than ever before if nuclear war is to be averted and peace secured". Following agreement on this statement, a telegram protesting at the decisions was also sent to Canada's Ministry of External Affairs by all members of the Canadian delegation at the meeting.

No action, however, was taken by the nine Soviet scientists who were already attending the meeting, and who continued to participate in the closed sessions.

At the end of the week-long meeting, the council endorsed a so-called "suffocation" strategy for limiting the spread of nuclear weapons that had first been proposed three years ago by Canadian prime minister Pierre Trudeau. Under this strategy, an immediate moratorium on the deployment of new weapons would be followed by agreements to limit weapons production and tests, a ban on all nuclear tests and a cutoff in the production of fissile material.

The statement said that the Soviet and US governments should "reaffirm their intention to maintain equal security at more stable and lower force levels". It also considered it essential that serious negotiations on limiting nuclear weapons in

Europe begin soon "before it is too late to set low limits", and in the context of moves that it said might destabilize the present balance of forces between East and West, suggested that highly accurate counter-strike missiles "are particularly dangerous since they create mutual fears of a first strike".

Soviet scientists at the meeting strongly denounced the United States for apparently dragging its feet over arms control negotiations. "The only obstacle on the way to arms control is the position of the United States," said Georgi A. Arbatov, director of the Soviet Institute for United States and Canadian Studies. The Soviet delegates resisted criticism of moves which had been taken by their own country.

One of the other proposals that the council agreed to support was for the United Nations to organize a global conference on international security. The council also advocated a "global approach" to the problem of future energy supplies and the potential conflicts that could arise over energy shortages.

"The general feeling is that it has been a very successful conference," said Mr William Epstein, the conference organizer and head of the Canadian delegation, adding that the refused visas had become "a relatively minor issue after a small flurry on the first day."

David Dickson

## Forensic science

### Evidence upheld

Dr Colin Horncastle, the British forensic scientist who was taken off casework after publishing what has been described as a "farcical" and "archaic" paper on toxicology, last week lost his appeal against the ruling of an industrial tribunal. The tribunal had upheld the decision of Dr A. S. Curry, controller of the Forensic Science Service, to take Dr Horncastle off casework, ruling that the offer of a teaching post meant that he had not been effectively dismissed. The Employment Appeal Tribunal found last week that the original judgement had not erred on points of law.

Dr Horncastle's is one of three recent cases in which senior forensic scientists have been dismissed. Last week, the Home Office announced that Dr Alan Clift, a principal scientific officer with the West Midlands forensic service, is to be compulsorily retired "on grounds of limited efficiency". Dr Clift has given forensic evidence on at least one occasion which has led to a wrongful conviction. The third case concerns a police surgeon who was dismissed for giving evidence for the defence.

The Home Secretary has announced that all Dr Clift's cases since 1966 in which the defendant had pleaded not guilty but was convicted are to be reexamined. And there have been calls for a full inquiry into the running of the Forensic Science Service.

Forensic scientists themselves seem to be divided in their opinions.

The case of Dr Horncastle, who worked in Chepstow at one of seven regional forensic laboratories, began after a paper he had submitted in 1973 to the journal of the British Academy of Forensic Science, *Medicine, Science and the Law*, was published in 1977. Dr Alan Curry, with the advice of six toxicologists, considered that the paper cast doubt on Dr Horncastle's competence to give evidence in court and that it was the product of a deranged mind. Called "Toxicology: quantitative aspects" (Vol. 17, No. 1, p.37), the paper relates the drug content of organs to dose and estimates time of death using a simple law of linear diffusion. The data are scattered, leading the author to dwell on the uncertainties of forensic science.

Dr Horncastle was first moved from casework to research. But two years later, after a poor assessment of performance, a retirement board recommended that he be offered a teaching post, which he turned down last year in favour of voluntary retirement.

Dr Horncastle has argued that the paper he published in 1977 was very similar to a talk he delivered at the Forensic Science Service's Central Research Establishment at Aldermaston in 1969, which aroused no adverse comment even though Dr Curry was chairing the meeting. He also points out that in the years between writing and publication of the paper there had been no complaints about his work, and that he might never have been aware of the strength of opinion had he not taken his case to the Industrial Tribunal.

Judy Redfearn

## Air pollution control

### Indoor hazards

#### Washington

The US research community seems to be on a collision course with the Reagan Administration over the need for further study of the health effects of indoor air pollution, ranging from radon emitted by building materials to the second-hand effects of cigarette smoke, and the formaldehyde used in foam insulation.

Mrs Anne Gorsuch, the new administrator of the Environmental Protection Agency (EPA), has apparently decided on major reductions in the agency's support for research into indoor air

pollutants in the 1982 fiscal year, which begins next month, and to eliminate the research programme the following year.

These decisions follow close on the heels of a report published by the National Academy of Sciences which claims that although indoor exposure can constitute an important fraction of the total exposure to many pollutants, it has been largely overlooked in research on the health effects of environmental pollutants. In some cases,

such as radon, the report says that there is an "urgent need" to study such health effects, since on the basis of known effects in miners exposed to radon and radon progeny at relatively high concentrations, "a plausible case can be made that a substantial fraction of the lung cancer incidence in non-smokers is due to the alpha-radiation dose to the respiratory tract epithelium from inhaled and deposited radon progeny particles".

The academy report, which was prepared for EPA by a committee of the National Research Council's board on toxicology and environmental health hazards, supports the conclusion of a report issued last year by the General Accounting Office, the investigatory arm of the US Congress, that indoor air pollution may pose a "potentially more serious health problem" than the degradation of outdoor air on which the federal government's clean-up efforts have so far been concentrated.

Other studies have reached a similar conclusion. A recent meeting of the public health committee of the New York Academy of Sciences reached a consensus view that "there are important and neglected disease consequences related to indoor air pollution" from causes that include viruses and bacteria, toxic gases, chemical radiation and particular matter.

The indications in Washington are that, far from increasing the budget for research into such effects, EPA and other agencies intend to cut back on research funds. Virtually all of EPA's present research into the potential effects of radon, for example, is being dropped.

Yet scientists with the General Electric Research and Development Center in Schenectady, New York, claim that there is "general recognition" by health physicists that the public receive greater exposure to radioactivity through "natural but controllable causes" in homes and other types of buildings than from the "hypothetical hazards associated with the generation of nuclear power".

The federal government has not ignored such warnings. For example, an inter-agency group set up two years ago to coordinate the work of the various agencies concerned with indoor air pollution published an inventory of present research in the field and the proceedings of a workshop on research needs, and is now working on an outline for future research priorities which may indicate how work should be distributed between the agencies.

Two obstacles stand in the way of such a plan. The first is an Administration zealously pursuing a desire to minimize the economic impact of health and environmental regulation. The second barrier is inter-agency tension caused by conflicting mandates. EPA, for example, estimates that the Department of Energy's programme for improving home insulation could cause between 10,000 and 20,000 additional lung cancer deaths a year due to

increased radon build-up. The department disputes these figures.

Such conflicts have inevitably created difficulties over research coordination. At one point, for example, the inter-agency committee was planning to suggest that EPA be appointed the principal agency for coordinating the federal attack on indoor air pollution.

However, this was apparently vetoed by the Office of Management and Budget, which was reluctant to commit the Administration to the substantial expenditures that an aggressive regulatory programme might entail, and sympathetic to the Department of Energy's arguments that it should be allowed to share lead-agency responsibilities.

The position of the new hierarchy at EPA on indoor air pollution research has yet to be officially revealed. Last month Representative Toby Moffett, chairman of the environment, energy and natural resources subcommittee of the House Committee on Government Operations, wrote to Mrs Gorsuch asking for details of the agency's plans and suggesting that, rather than cutting back, "EPA should instead be expanding its research effort".

No reply has yet been received from Mrs Gorsuch. **David Dickson**

## British Association

### Royal occasion

The 150th anniversary meeting of the British Association passed off decorously enough last week in the splendid architectural setting of the city of York. The Duke of Kent delivered a forthright defence of science against its critics in one of the loveliest of British cathedrals, York Minster. A symposium of distinguished academics surveyed the past 150 years in their own subjects with a little less sense of occasion than their written texts suggested would be appropriate (see *Nature* 3 September, p.13), half of them in the opulent academic setting of the University of York, increasingly regarded as a monument to the time when the cost of university education was regarded as no impediment to its indefinite expansion; a symposium of speakers with contrasting views held forth on the prospects of nuclear war in Europe; the association failed to come to a decision about the appointment of a secretary; and none of those among its members questioned knew why a 150th birthday party should be called a "sesquicentenary".

The Duke of Kent, a Yorkshireman by marriage but not on that account qualified to play cricket for the county of Yorkshire, surprised his audience by the zeal of his commitment to the cause of unfettered science. Imagine, he said to his audience, a world without electricity. Would we like that? Would we be any happier? So should we not take with a pinch of salt the siren calls of those who say that we would all be

## Ariane delayed

The fourth and final test flight of Ariane, the European Space Agency's rocket launcher, will probably be delayed beyond mid-November by a problem with its payload, the maritime communications satellite, Marecs. Mechanical interference between the satellite's antenna and body is causing passive inter-modulation of radio waves at high frequencies, according to British Aerospace, prime contractor for the satellite. The problem, not uncommon in telecommunications satellites, has apparently been solved under ambient conditions but tests still need to be done in a thermal vacuum chamber. The launch is now unlikely before early December. **Judy Redfearn**

more human if Faraday and Maxwell had never lived? Some asked themselves who the Royal Duke was getting at; everybody agreed that it was good stirring stuff — stuff calculated to help them endure the city of York's boxed lunch.

The nuclear symposium was, in its way, a daring innovation on the part of the association. Mr Edward Thompson, the historian, made an arresting speech on behalf of the cause of non-governmental efforts in support of European nuclear disarmament; Dr David Owen, Foreign Secretary in the previous British government and now one of the founders of the Social Democratic Party in the United Kingdom, came out in favour of a nuclear-free zone in central Europe. As has been the association's habit for the past 150 years, there was too little time for discussion by the time that every listed speaker had had his (*sic*) say.

Otherwise, the association provided for its members the usual miscellaneous range of conversational gambits. Did you know that there must be something wrong with the textbook explanations of geomorphism and phototropism (the phenomena by which plant roots grow down and supra-terrestrial parts of plants grow towards the Sun respectively)? Did you know that not everybody accepts the cataclysmic explanation of the transition from the Cretaceous to the Tertiary? With a whole day given over to the formal birthday celebrations, it is perhaps no wonder that some members were disappointed that there had been so little into which to sink their teeth.

The higher politics of the association are increasingly unbelievable. On occasions such as the annual meeting, members of several tiers of committees have a chance to say what they think should happen to the association. Their outstanding problem is the appointment of a secretary, essentially their chief executive officer. Apparently, not decision has been reached except that, if a suitable candidate "comes along", he (or she) will be appointed.



# CORRESPONDENCE

## Diet-induced polyuria

**SIR** — We have observed a trait of C3H/He mice which makes them unsuitable for some experiments but which is of intrinsic interest. While testing a highly purified, minimal-antigen diet for acceptability by mice of this strain, we noticed that they produced vast quantities of urine, many excreting their body weights in urine daily.

Female C3H/He mice, weighing 15g, drank 7 ml water and excreted 2 ml urine daily when fed BP Nutrition Diet 3 (a typical breeding diet). After 3 days on the test diet the same mice drank 20 ml water and excreted 15 ml urine daily. When returned to the stock diet, they reverted to normal.

We have successfully maintained without polyuria strains NMRI and BALB/c on this test diet, so it seems likely that C3H/He mice have a genetically determined lesion in their regulatory mechanism for water balance. Although the composition of the diet is complex (49 ingredients) it is based on the National Research Council requirements for rats<sup>1</sup> and contains 13 per cent amino acid mixture, 3.6 per cent corn oil, 78 per cent glucose, 3.9 per cent mineral mixture and 1.1 per cent vitamin mixture.

As we ourselves are not studying water balance, we hope that this report will stimulate further investigation of this phenomenon by others with whom we would be pleased to cooperate.

A. WISE  
D. TUCKER

MRC Laboratory Animals Centre,  
Carshalton, Surrey, UK

1. National Research Council, *Nutrient Requirements of Laboratory Animals* (National Academy of Sciences, Washington DC, 1978).

## A correction

**SIR** — An article by Jasper Becker (*Nature* 20 August, p.665) describes the German Cancer Research Institute in Heidelberg and the politics at that institution. The third paragraph states, "The attack soon broadened to Neurath's qualifications and it turned out that he had in fact previously only been scientific advisor to the University of Seattle". I would like to set the record straight regarding this inaccurate reporting.

Dr Neurath was professor and chairman of the Department of Biochemistry at the University of Washington for 25 years. He has authored or co-authored over 350 manuscripts published in major scientific journals. Furthermore, he has been editor of *Biochemistry* during the past 20 years and editor or co-editor of a number of scientific books. Since 1961, he has been a member of the National Academy of Sciences in the United States. He was also the associate director for research at the Fred Hutchinson Cancer Research Center here in Seattle. Thus, for your article to state that he had in fact previously only been scientific advisor to the University of Seattle is totally erroneous. A simple phone call to any biochemist in the world could have clarified this poor reporting.

EARL W. DAVIE

University of Washington,  
Seattle, USA

We apologize to Dr Neurath and the University of Washington for this error — Editor, *Nature*.

## Resistant cattle

**SIR** — I read with interest your comments on the plans for a new research institute in the Gambia to investigate why N'Dama cattle in Africa seem to be more resistant to trypanosome infection than Zebu cattle (*Nature* 9 July, p.102).

At least two new institutions have set up with the same objectives and are now operational. One is the Centre d'Elevage et de Recherche sur la Trypanosomiase et la Trypanotolérance located in Avetonou, Togo, which is sponsored by the German Agency for Technical Cooperation (GTZ) and the Togolese government. Several thousands of pedigree N'Dama, as well as Baoulé, Zebu and "local breed" cattle are available and the emphasis is on breeding cattle resistant to trypanosomiasis and making them available to local farmers. The other is the Centre de Recherches sur les Trypanosomoses Animales in Bobo-Dioulasso, Haute-Volta, sponsored by GTZ and the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux (Maisons-Alfort, France). Here the emphasis is on more basic research in immunology, biochemistry and genetics. There are also excellent tsetse fly raising facilities.

Research on some genetic markers of trypanotolerance, including erythrocyte groups, glucose-6-phosphate dehydrogenase, haemoglobins and transferrins, is near completion. Characterization of genetic markers for the identification of resistant cattle using monoclonal antibody technology is under way.

The two centres are working in close cooperation and have access to further material through a network of institutions and field stations in Togo, Ivory Coast, Upper Volta and Mali, hopefully to be extended to other geographical areas.

We would gladly provide technical information to anyone interested.

GEORGES E. ROELANTS

Bobo-Dioulasso,  
Upper Volta

## Random diets

**SIR** — Dr Yudkin<sup>1,2</sup> agrees with the recent report of the Royal College of Physicians<sup>3</sup> that, while there is some evidence that dietary fibre may reduce the risk of colon cancer, it falls far short of proof beyond reasonable doubt. Drs Weisburger and Reddy<sup>4</sup>, on the other hand, believe that the present data base is adequate to conclude that fibre-rich bread and cereal "have every chance of reducing the risk of colon cancer". That correspondence<sup>2,4</sup> illustrates yet again how difficult it is to gain reliable information about the health effects of dietary factors. Our recent review<sup>5</sup> of the causes of cancer, while concluding that on present evidence it was very reasonable to hope that certain (though perhaps not all) types of dietary fibre were importantly protective, emphasized that it was also reasonable to remain unconvinced, and recommended that the practicability of randomly controlled interventions be investigated.

One of the currently favoured hypotheses is that certain types of fibre affect the *later*

stages of the process of carcinogenesis, in which case (to judge by the rapidity with which measurable effects of cessation of smoking are evident) there should be a substantial reduction in risk during a period only 5–9 years after switching to a high-fibre diet, so it might just be possible to obtain reliable evidence, acceptable to all parties, by means of a really large randomized trial lasting for about 10 years. However, even among people aged about 70, only about 1 per cent will develop cancer of the large intestine during a given five-year period. So, to observe a few hundred disease onsets between years 5 and 9 in such a study, a few tens of thousands of people would have to be randomized. The practical problems of such an enterprise are formidable, though they may not be insurmountable as long as the people to be randomized are selected appropriately (to judge by the degree of continuing collaboration among some thousands of members of the British medical profession in a randomized evaluation of treatment of the doctors with prophylactic aspirin). The feasibility of a randomized evaluation of particular types of dietary fibre (and of various other dietary hypotheses) is a matter that can be resolved not by assertion but by pilot studies, in carefully selected populations, of the number of subjects that can be recruited, the cost per subject (which must, by simplicity of design, be kept low), and the degree of compliance that can be attained in a large randomized trial.

If some such randomized studies are practicable then they could lead to an unequivocal resolution of many dietary hypotheses, relating to both neoplastic and vascular disease, that would otherwise be subjected to indefinite dispute. Although randomized evaluation of the top ten dietary hypotheses (including, hopefully, the role of various types of dietary fibre) may yield only one or two findings that are clearly positive, the public health implications of having a few pieces of *reliable* dietary information to communicate would easily justify the costs of several such trials, even if most found no differences.

Observational epidemiology does strongly suggest that some unidentified dietary factors are important determinants of cancer risks<sup>5</sup>, but although observational epidemiology alone has yielded proof beyond reasonable doubt of the main effects of tobacco, alcohol, radiation and certain occupational and reproductive factors<sup>5</sup>, the complexity of diet and the strong correlations that commonly exist between different dietary factors mean that equally reliable evidence about diet may be difficult to attain without large, simple, long-term randomized studies.

RICHARD PETO

Radcliffe Infirmary,  
Oxford, UK

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## NEWS AND VIEWS

# Droughts, severe winters and sudden stratospheric warmings

from Dennis Hartmann

CLIMATOLOGISTS and meteorologists have long puzzled over the causes of large anomalies in the weather. One does not need a particularly good memory to recall such anomalies: the summer drought in Great Britain in 1976 and the combined American western drought and eastern cold spell during the 1976–77 winter are good examples. These events are as important today as they have ever been, both because of the demand on energy and water resources and because agriculture has been planned to be efficient in average weather conditions. If the anomalies could be anticipated, the social and economic disruption they cause could be greatly lessened.

It is useful to think of the state of the atmosphere as composed of the average around latitude circles (the zonal mean) and the deviation from this average (the eddy or wave component)<sup>1</sup>. Most of the year-to-year variability of the average weather in a particular region can be associated with shifts in the position and intensity of quasi-stationary, large spatial scale waves forced by localized heat sources and mountain ranges. These waves are known as planetary waves and need to be distinguished from the smaller-scale transient storms which produce most of the precipitation in mid-latitudes. The paths which the transient storms follow during their life cycles are determined by the flow pattern composed of the zonal mean and the quasi-stationary planetary waves. Changes in the planetary wave structure can produce droughts by steering storms away from a region. A climatology of planetary waves, based on a Fourier analysis of the height of the 500 mbar pressure surface, has recently been presented by Reiter and Westhoff<sup>2</sup>. They point out that the amplitude and degree of stationarity of particular Fourier components at particular latitudes vary both with season and on an interannual basis.

It is known that there is some degree of organized structure to the interannual variability in the wind and temperature fields associated with droughts and cold

winters in particular regions. A comprehensive description of the patterns which appear in the Northern Hemisphere during winter has recently been given by Wallace and Gutzler<sup>3</sup>. Some of these patterns suggest direct links between anomalies in the tropics and anomalies in mid-latitudes, particularly for the western North American region<sup>4</sup>. Hoskins and Karoly<sup>5</sup> have applied linear wave theory to the horizontal propagation of planetary Rossby waves on a sphere and have been able to reproduce the general shape of the anomaly patterns found in the observations. The theory indicates that the group velocity of the wavetrains corresponding to the observed anomaly patterns which extend from the tropics to mid-latitudes is such that energy flows from the tropics towards higher latitudes. The theory also predicts that, in the absence of dissipation, the amplitude of stationary waves forced in the tropics will increase along the wavetrain towards higher latitudes. This result, combined with the fact that very large localized latent heat release is common in the tropics, suggest that it may be easier to force mid-latitude anomalies from the tropics than from within the mid-latitudes themselves. This conclusion depends heavily upon the amount of dissipation to which the planetary waves are subject and also upon the details of the planetary wave behaviour where the zonal mean wind approaches zero, at which point the linear theory may not be adequate. A satisfactory nonlinear theory for the behaviour of planetary waves near a surface of zero zonal mean wind is not yet available. Nonetheless, the existence of preferred patterns for the interannual variability of climate and the apparent success of linear theory in producing some of these patterns has led to renewed hope that interannual variability may eventually be understood and perhaps forecast.

*Dennis Hartmann is in the Department of Atmospheric Sciences, University of Washington, Seattle.*

Recent observational and theoretical advances have also been made in the area of stratospheric dynamics, where planetary waves are again of major importance. The stratosphere, the region from approximately 15 to 60 km above the Earth's surface, contains the majority of the atmosphere's ozone, which protects the biosphere from most of the Sun's harmful ultraviolet radiation. The amount of ozone in the stratosphere is controlled both by photochemistry and by transport by atmospheric motions. A Fourier analysis around latitude circles shows that the majority of the wave energy in the stratosphere is contained in the first three Fourier components. These planetary waves are known to originate in the troposphere, and are, in fact, the same waves which account for the east–west climate variation at the surface.

The upward propagation of these waves from the troposphere to the stratosphere can be understood in terms of the linear theory of planetary Rossby wave propagation through the medium defined by the zonal mean wind and temperature distribution. Our knowledge of the stratosphere has been greatly increased with the advent of satellite-based indirect observations, beginning with the Selective Chopper Radiometer developed at the Department of Atmospheric Physics of Oxford University<sup>6</sup>. The paper by Chapman and Miles<sup>7</sup> in this issue of *Nature* describes the use of conventional and satellite observations to trace the upward and meridional flow of planetary wave energy from the troposphere to the stratosphere. They show that the general behaviour of the energy flux vectors is in good agreement with linear wave propagation theory.

The upward propagating planetary waves can alter the zonal mean structure through which they propagate. The most spectacular example of the effect of upward propagating waves on zonal mean conditions in the stratosphere is the sudden stratospheric warming phenomenon<sup>8</sup>. In winter the polar temperatures in the stratosphere are generally much colder

than the equatorial temperatures. During sudden warmings, which occur in mid-winter in the Northern Hemisphere roughly every other year, over the period of a few weeks the polar temperatures increase by as much as 60 K and the normal west to east zonal mean wind is reversed. During one of these events the total ozone column in middle and high latitudes is greatly increased. Clearly, such dramatic events call for an adequate theory of wave-mean flow interaction. Recently Andrews and McIntyre<sup>9,10</sup> have advanced the theoretical understanding of wave-mean flow interaction by introducing generalized lagrangian mean and transformed eulerian mean forms of the equations of motion. The transformed eulerian mean

formulation provides a useful framework for diagnostic studies of wave-mean flow interaction, wherein changes in the zonal mean flow are related to the divergence of the so-called Eliassen-Palm flux<sup>11</sup>. The Eliassen-Palm flux vectors would appear much like the energy flux vectors shown by Chapman and Miles but are a measure of wave action flux rather than wave energy

flux. These vectors are parallel to the group velocity for stationary, conservative waves on a constant mean flow. Recent studies with observations and numerical simulations of sudden warmings suggest that the vectors, which normally point upwards and equatorwards, point upwards and polewards when a warming is taking place<sup>12,13</sup>. □

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## Looking for genomic changes in cancer cells

from B.A.J. Ponder

MUCH of cancer research is aimed at discovering how a normal cell turns into a cancer cell. A straightforward approach is to compare the cancer cell with the normal cell from which it was derived and look for informative differences. So far, most effort has gone into comparing cellular phenotypes, because techniques have been available to make the comparisons. With the development of recombinant DNA technology, a direct attack on genomic differences between normal cells and cancer cells should also be possible. A report, by Peter Humphries, of one of the first attempts to use this approach appears on p.146 of this issue of *Nature*.

Those who wish to take the genomic route to comparing normal cells and cancer cells have several options. If they wish to search directly for sequences which may be involved in the origin of a particular cancer, there are in principle at least three possibilities. By using chromosome sorting and the construction of cloned genomic libraries they can examine the sequence changes in areas of gross chromosomal abnormality which are known to be associated with particular tumours: for example, the 13q- deletion in retinoblastoma<sup>1</sup> or the 22→9 translocation in chronic myeloid leukaemia<sup>2</sup>. By gene transfer experiments, either by transfection<sup>3</sup> or by using retroviruses as vectors<sup>4</sup>, they may hope to identify critical cellular genes for cancer formation, and then to construct DNA probes to compare the arrangement of these sequences in normal cells and their malignant counterparts. Finally, they may hope to identify these critical genes in certain human inherited cancer syndromes by classical genetic linkage studies using either known polymorphic loci — as has been used in breast cancer<sup>5</sup> — or polymorphisms in DNA base sequence which are reflected in restriction enzyme cutting patterns, a potentially much more powerful approach

advocated by Botstein and his colleagues<sup>6</sup>.

The alternative is to fish for changes in DNA sequence organization without any particular chromosomal site in mind, in the hope of finding evidence of genetic rearrangement which might be of importance in the aetiology of cancer. The case for the importance of such rearrangements has recently been argued by Cairns<sup>7</sup>. The middle or low copy number repetitive sequences might be a good place to look, because changes in these sequences might be relatively easy to detect, and because what we know of transposable genetic elements in other systems<sup>8,9</sup> suggests that these sequences are good candidates to be involved in the kinds of genomic rearrangements we are after. This is the approach taken by Humphries with results which are still very preliminary, but which will be intriguing if they can be extended.

The DNA probe he used to search for genomic variation is a complex one prepared from an *EcoRI* digest of human fetal liver DNA. It is a mixture of 100 DNA fragments containing unique or low copy number repeated sequences which have in common that they come from a region of the fetal liver cell genome which lies within either a 14 kilobase (kb) or a 50 kb *EcoRI* fragment. The probe is used to compare the arrangement of its complementary sequences in *EcoRI* digests of DNA extracted from normal gut mucosa and in cancer arising from it. The known polymorphism in DNA sequence between individuals<sup>10</sup> means that the pattern of *EcoRI* fragments obtained might vary from one individual to another, so that the cancer and normal tissue which are to be

compared must be taken from the same individual. Then, any differences in the fragment pattern should indeed reflect differences between normal and cancer cells.

The first result which was obtained, with DNA from normal gut mucosa, is in many ways the more interesting. Several *EcoRI* fragments smaller than 14 kb are seen to hybridize with the probe sequences. Densitometer tracings suggest that most of the fragments are different in the samples from each of the five individuals shown. Samples of colonic mucosa from two individuals are similar, but that from a third is quite different; the normal rectal and stomach mucosa also give different patterns. This result indicates that some of the sequences which were present in 14 kb and 50 kb *EcoRI* fragments in the fetal liver DNA also occur in the DNA samples from gut mucosa within different arrangements of *EcoRI* sites. It is not clear what pattern was obtained by hybridizing against the total fetal liver DNA from which the probe was made. The sequences which are hybridizing appear to be multiple copy sequences, but presumably the *EcoRI* fragments within which they lie may be unique. A crucial question is to what extent the differences between the various samples reflect differences between tissues or between individuals. Because polymorphic differences in DNA sequence between individuals are already known to exist, one might assume the latter; but the point is not proven. Interpretation is made more complicated because one cannot be sure that the same DNA sequences out of the 100 or so in the probe are hybridizing in each case. Even with these reservations, the results imply a striking degree of polymorphism of *EcoRI* sites around these repeated DNA sequences. While we know so little about the organization of such sequences in the mammalian genome, these

B.A.J. Ponder is a C.R.C. Fellow and Honorary Consultant Physician at the Institute of Cancer Research and Royal Marsden Hospital, Sutton, UK.



seem important points to pursue. In particular, for the comparison with DNA sequences from the cancer, the question of tissue differences is crucial; one has first to be sure that one is indeed comparing comparable cells. If there is no difference in sequence organization between different cell types in an individual, the problem disappears — but if there are, one cannot be sure that DNA from the mixture of cells in normal mucosa is comparable with that from a tumour, however adjacent.

Leaving these objections aside, the results obtained with the *EcoRI* digests of tumour DNA show that some of the hybridizing sequences found in DNA from normal mucosa are no longer present in fragments less than 14 kb long in an *EcoRI* digest of the tumour DNA. Degradation of the DNA during extraction from the tumours is controlled for by recovery of DNA in the satellite bands. One possibility is that the hybridizing sequences have been lost altogether, perhaps as a result of loss of certain chromosomes in the tumours (hyperdiploidy or pseudodiploidy is quite compatible with the loss of specific chromosomes, as indeed has been described in colonic polyps<sup>11</sup>). An alternative, more interesting but perhaps

more difficult to envisage, is that they have been subject to some genomic rearrangement which has separated them from their flanking *EcoRI* sites. In that case one might expect to see them hybridize in new fragments. Even if one of these explanations of the results is correct, the relationship of these events to those which lead to the development of the cancer remains, as Humphries points out, to be established.

No doubt this is the first paper of many that will use this new approach. If these papers have to tell us something about the sequence organization of the human genome and its variations before they start to tell us about cancer, they will be none the worse for that. □

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## Symbiotic stars

from David Allen

A workshop held in June at the Joint Institute for Laboratory Astrophysics, University of Colorado, was the first meeting to be devoted exclusively to symbiotic stars. The term symbiotic was adopted in the astronomical context by Paul Merrill almost 40 years ago. The symbiotic stars exhibit optical spectra which combine two temperature regimes. Emission lines of uncommonly high excitation suggest temperatures of several hundred thousand Kelvin; yet the underlying stellar continuum is generally that of an M-type giant star of 3,500 K or less. In Merrill's time a handful of symbiotic stars was known; today the tally exceeds 100. Though not resolvable as binary stars, their spectra often display radial velocity variation consistent with binary motion.

An impressive array of observational data was presented at the workshop, from the X-ray to the radio domains. Perhaps the most valuable new data are those obtained by the International Ultraviolet Explorer (IUE) satellite, described by Michalitsianos (Goddard Space Flight Center) and by Kafatos (George Mason University, Virginia). The IUE data show

symbiotic stars to be a diverse family characterized by extraordinarily strong emission lines. Although the array of emission lines recorded by IUE is much the same in all symbiotic stars, the velocity profiles of the lines vary greatly both from star to star and from line to line in a particular star. Some lines are simple narrow Gaussians whereas others exceed 1,000 km s<sup>-1</sup> in width and are dissected by overlying absorption. Moreover, the UV continua also range considerably, corresponding to implied temperatures of 10,000–200,000 K. Somewhat higher temperatures were revealed by X-ray detections made with the Einstein satellite by Anderson (Washburn Observatory, Wisconsin) and by Allen (Anglo-Australian Observatory). Approximately 10 per cent of symbiotic stars surveyed were detected by Einstein, all with extremely soft spectra corresponding to temperatures of slightly less than 10<sup>6</sup> K. The situation is again complicated by a single example, known for some years, which is an intense, pulsed hard X-ray source.

The gas density derived from optical and UV lines indicates from 10<sup>11</sup> to 10<sup>16</sup> electrons cm<sup>-3</sup>. In some symbiotic stars there is a bulk outflow of gas so that the nebula is sufficiently extended to radiate detectable thermal Bremsstrahlung in the radio waveband. In reviewing the radio

data, Hjellming (National Radio Astronomy Observatory) pointed out that the changing radio spectrum can yield information on past variations in the mass-loss rate. In particular, some systems have reduced their mass-loss rates and so generated a hollow nebula. Graphic evidence of this was provided by Newell (National Radio Astronomy Observatory) and Hjellming, using the Very Large Array telescope. They showed a radio map of V1016 Cygni only 0.3 arcs in diameter. The hollow centre could be seen, together with a suggestion of preferred ejection into two opposing regions.

The variability of symbiotic stars received considerable attention. Many specimens erupt at irregular intervals, brightening by an order of magnitude at optical wavelengths, but by much more in the UV. Such eruptions usually last a few months. Other symbiotic stars have exhibited a 1,000-fold increase in brightness from which the fading requires decades; yet others appear to be in permanent outburst. In a few cases repetitive fading points to the presence of eclipses, and thus of two components in mutual orbit. The orbital periods are generally a few years. Periods in the range 1–2 yr are also seen in the IR brightness in some cases: such variations are interpreted as the Mira-like variations of the cool giant.

Undaunted by the bewildering array of observational material, the theoreticians provided credible explanations for the phenomena of symbiotic stars. There was general approval for the model proposed by Bath (University of Oxford), who envisages a cool giant star shedding material onto a companion star, primarily by Roche Lobe overflow. The gas falling onto the companion releases gravitational energy at a temperature suitable for exciting the observed emission spectrum. In most cases the gas is transferred via an accretion disk. Variations in the transfer rate would be expected because of the unstable nature of cool giants; these changes account for the outbursts. The companion need be no more exotic than a solar-type main sequence or subdwarf star. Indeed, Robinson (University of Texas at Austin) provided strong arguments for discounting white dwarfs as the companions in most cases. In only one case, mentioned above, does the hard X-ray flux indicate accretion onto a neutron star. In the case of CI Cygni, analysis of the eclipse by Webbink (University of Illinois) clearly indicated the existence of an accretion disk around a main sequence star during the 1975 outburst.

Variants on Bath's model appear tenable in some cases. One interesting suggestion by Willson (State University of Iowa) and Wallerstein (University of Washington) and by Kwok (Herzberg Institute, Canada) explained the excitation of the emission lines by the collision of winds from the two

stars. The thermonuclear flash which occurs when hydrogen-rich gas is accreted onto white dwarf stars, and which accounts for conventional novae, may also be relevant to some symbiotic stars. Identification of the cool star itself as the source of the high-excitation emission lines has not been ruled out, for the binary interaction may inject energy into the star's corona.

Parallels between symbiotic and other interacting binary stars were sketched by Stencel (Joint Institute for Laboratory Astrophysics, Boulder) and by Plavec (University of California, Los Angeles). A precise definition of symbiotic stars does not seem possible, for binaries exist with a range of degree of interaction. Perhaps the only defining characteristic is that one member is a cool giant star. □

## Marsupials: alternative mammals

from Marilyn B. Renfree

MARSUPIALS have come to be regarded as second class mammals, partly because of their unusual mode of reproduction, and partly because they were discovered long after the eutherians (or 'placental' mammals) and were all lumped into one group, the Order Marsupialia<sup>1</sup>. Today, marsupial biologists recognize at least three, and commonly four, orders of marsupials and take exception to statements of their supposed inferiority.

As recently as 1977, *Nature's* article "Why marsupials can't win"<sup>2</sup> aired many commonly held misconceptions and it took Taylor and Padykula's<sup>3</sup> defence in the same journal to redress the balance. In North America, the 'primitive' (that is, unspecialized) Virginian opossum is currently extending its range north and south into the territories of the 'advanced' eutherians. Furthermore, there is no sign of present day South American marsupials being squeezed out by their eutherian neighbours. Even in Australia, the home of so many marsupial species, they coexist in harmony with numerous indigenous small eutherian rodents. And if the European rabbit, fox and cat have flourished in the wild following their introduction to Australia, it should also be remembered that Bennett's wallaby has bred for decades as a feral animal in England. The brush-tailed possum introduced to New Zealand from Australia has become as great a pest there as the rabbit is in Australia.

Recent studies suggest that far from being 'inferior' to eutherians, marsupials have evolved an alternative and very successful reproductive strategy. The first description of reproduction in an Australian marsupial (the tammar wallaby, *Macropus eugenii*) was given by Francisco Pelsaert, captain of the Dutch ship *Batavia* which was wrecked on the Abrolhos Islands in N.W. Australia in 1629.

Their generation of procreation is Very Miraculous, Yea, worthy to note; under the belly the females have a pouch into which one can put a hand, and in that she

has her nipples, where have discovered that in there their Young Grow with the nipple in mouth, and have found lying in it (the pouch) some Which were only as large as a bean, but found the limbs of the small beast to be entirely in proportion, so that it is certain that they grow there at the nipple of the mammal and draw the food out of it until they are big and can run.

However, it was to take another 300 years before scientists confirmed that the marsupials do indeed have a unique mode of reproduction, with the investment in lactation rather than gestation.

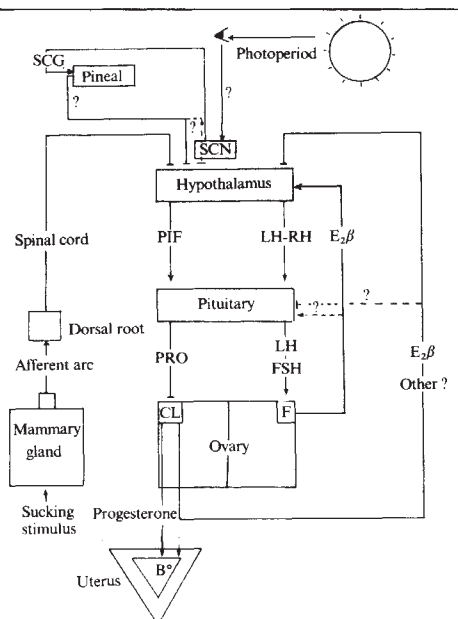
Their lactational physiology is certainly sophisticated, and we still do not understand how they manage simultaneously to suckle young of different ages from adjacent mammary glands secreting milk of different compositions. An interesting recent observation was that the gland supporting the newborn young is much more sensitive to oxytocin than the gland supporting the young-at-foot<sup>4</sup>. Were this not the case, the small pouch young permanently attached to the teat would produce a flood of milk from

the other gland every time it sucked, until it would eventually drown in a pouch full of milk destined for its older sibling!

Since Sharman's 1955 description of embryonic diapause in a small wallaby, the quokka *Setonix brachyurus*<sup>5</sup>, much attention has been focused on the macropodid (literally 'big footed') marsupials (the kangaroos and wallabies). In particular, the tammar wallaby, which breeds well in captivity, has proved most amenable to experimental manipulation. The studies of Tyndale-Biscoe and colleagues in Canberra have revealed much about its reproduction, highlighting the central role of the corpus luteum. We now know more about the reproductive physiology of the tammar than any other marsupial.

Pregnancy does not interrupt the periodicity of oestrus, so female tammars conceive again 24–48 h post-partum<sup>6</sup>. Curiously, sperm do not pass beyond the cervix of the parturient uterus, but rapidly reach the uterine lumen of the entirely separate, non-gravid side<sup>7</sup>. Ovulation alternates between the left and right ovary so that new follicular development occurs during pregnancy in the ovary contralateral to the gravid uterus. Thus there seems to be a local, unilateral inhibition of follicular development in the ovary containing the corpus luteum. The new embryo resulting from the post-partum mating grows to about 100 cells, enters and remains in embryonic diapause so long as there is a young sucking in the pouch<sup>8</sup>. The blastocyst will stay in diapause provided the corpus luteum remains quiescent; the activated corpus luteum is needed to reactivate the embryo<sup>9</sup>. The corpus luteum itself is held quiescent by prolactin; hypophysectomy removes this inhibition and the corpus luteum hypertrophies and becomes fully secretory<sup>10</sup>. Injections of prolactin after hypophysectomy will maintain the delay for the duration of the prolactin treatment<sup>11</sup>. Similarly, treatment

Hypothetical scheme showing pathways for the control of quiescence in the tammar wallaby (redrawn with additional information from Renfree, M.B. *Nature* 278, 549; (1979). B, blastocyst; CL, corpus luteum;  $E_2\beta$ , oestradiol-17 $\beta$ ; F, follicle; FSH, follicle-stimulating hormone; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; PIF, prolactin inhibiting factor; PRO, prolactin; SCG, superior cervical ganglion; SCN, supra-chiasmatic nucleus.  $\uparrow$ , Stimulation;  $\downarrow$ , inhibition.



Marilyn B. Renfree is Associate Professor in Reproductive Biology, Murdoch University, Western Australia.



of lactating wallabies in diapause with the dopamine agonist, 2-bromo  $\alpha$ -ergocriptine, will depress prolactin sufficiently to allow the corpus luteum to escape from this inhibition, and 27 days later a newborn young can be found in the pouch beside its older sibling<sup>6,11,12</sup>. Prolactin apparently acts directly on the luteal cells to exert this inhibitory effect because the membrane fraction of the quiescent corpus luteum contains highly specific receptors for prolactin, which are present in greater concentrations than in any other tissue in the tammar including the lactating mammary gland<sup>13</sup>. However, it seems unlikely that prolactin has a direct inhibitory effect on steroidogenesis, as prolactin added to corpora lutea *in vitro* does not alter progesterone production<sup>12</sup>. Since the pituitary is not necessary for corpus luteum activation, it was not surprising to find that LH added to the cultured tissue had no effect on steroidogenesis, and it seems that the tammar corpus luteum has no receptors for this gonadotrophin (F. Stewart and C.H. Tyndale-Biscoe, Personal communication).

Gonadotrophins are essential in follicular growth and development, since hypophysectomy causes complete follicular atrophy<sup>10</sup>. There is a marked surge in luteinizing hormone secretion 0–8 h after the onset of oestrus<sup>14</sup>, and if this is prevented by the administration of anti-LHRH antiserum, ovulation is inhibited (R.V. Short, M.B. Renfree & A.P. Flint, unpublished).

Oestradiol-17 $\beta$  has a negative feedback effect on pituitary gonadotrophin secretion if injected in low doses, but will induce a LH discharge if given in amounts of 10  $\mu$ g kg<sup>-1</sup> or greater<sup>15</sup>. It should be remembered that during normal pregnancy, follicles develop in the ovary contralateral to the active corpus luteum

about the time when progesterone concentrations are high and the corpus luteum has achieved maximum development. But paradoxically, it is the quiescent corpus luteum which is responsible for the suppression of ovulation during diapause: after luteectomy, even in the continued presence of a sucking pouch young, follicles grow and animals return to oestrus 11 days later<sup>11</sup>. This follicular growth can be inhibited by oestradiol-17 $\beta$  injections but not by progesterone<sup>15</sup>.

There is another aspect of tammar reproduction which makes it a valuable model for understanding control of mammalian reproduction in general. As well as having a period of lactational diapause during suckling, the tammar has superimposed on this lactational control a seasonal one. In the first half of the year, loss or removal of the pouch young results in hypertrophy of the corpus luteum and activation of the diapausing blastocyst, but after the winter solstice in June this procedure no longer has any effect and the blastocyst remains at 100 cells<sup>8</sup>. Photoperiod is the controlling factor<sup>16</sup>, and in the wild all wallabies in diapause

spontaneously reactivate their blastocysts after the longest day on December 22<sup>9,12</sup>, so that there is synchronization of births in late January. Prolactin concentrations are highest during seasonal quiescence and this is also the only time when bromocriptine fails to cause a resumption of embryonic development<sup>12</sup>. Work to clarify the seasonal control of reproduction is continuing; the pineal gland is clearly implicated<sup>17</sup>.

The tammar wallaby has many unusual and complex features in its mode of reproduction, and yet, because of the temporal separation of ovulation, diapause and implantation, and the existence of both a lactational and a seasonal inhibition of reproductive activity, provides an intriguing model to study. Because of the importance of lactation in the marsupials, prolactin seems to have become very important in their reproduction; its secretion can be modulated by the sucking stimulus, or by day length, as in eutherians, but in the tammar at least, it has assumed a new role as the key pituitary hormone in control of the corpus luteum, and hence the regulation of embryonic diapause. □

## The physics of sunspots

from Lawrence E. Cram and John H. Thomas

UNDERSTANDING sunspots has proved to be one of the greatest challenges in astrophysics. The study of sunspots has inspired the development of many ingenious and precise methods of measuring velocity and magnetic fields of the Sun, and on the theoretical side has been fundamental to many advances in basic magnetohydrodynamics. The first meeting in more than a decade to be devoted entirely to sunspots was held recently at Sacramento Peak Observatory in southern New Mexico\*.

P. McIntosh (NOAA, Boulder) summarized the structure and evolution of sunspots and sunspot groups, based on his observations of thousands of spot groups in connection with his work on solar forecasting. The formation of a sunspot group often occurs through the coalescence of a series of several (three to six) bipolar pairs that emerge at approximately 24-hour intervals. Small spots of leading polarity are often arrayed in an arc, which later collapses into a single, symmetric leading sunspot. Spots of following polarity are generally irregular in structure and shorter lived. Although most sunspots live for less than one solar rotation period ( $\sim 28$  days),

some live much longer, and an exceptionally stable spot that first appeared in August 1966 survived for over five rotation periods. Long-lived sunspots generally have a slower rotation rate than is typical for their latitude. Sunspots tend to appear at certain sites on the Sun and these sites may persist for several years. Synoptic observations of spots and other magnetic field tracers show that the active sites often rotate with periods significantly different from the surface plasma rotation rate.

According to P.R. Wilson (University of Sydney), the main problems to be solved in the theory of sunspots include explanations of the stability of the magnetic field configuration in a spot, the coolness of a sunspot and the disposition of the energy deficit, the evolution of sunspot groups, and the occurrence of a few very long-lived spots.

Wilson discussed the merits of three specific sunspot models, those of Piddington (*Astrophys. Space Sci.* **34**; 347, 1975), Parker (*Astrophys. J.* **230**; 905, 1979) and Meyer, Schmidt, Weiss and Wilson (*Mon. Not. R. astr. Soc.* **169**; 35,

Lawrence E. Cram is Associate Director of Sacramento Peak Observatory, Sunspot, New Mexico and John H. Thomas is Professor of Mechanical and Aerospace Sciences, University of Rochester, New York.

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\*The summer workshop on 'The physics of sunspots', organized by the L.E. Cram and J.H. Thomas, was held at Sacramento Peak Observatory, Sunspot, New Mexico on 14–17 July 1981.

1974). Piddington's model is based on twisted 'ropes' of magnetic flux, where the twist is essential to the stability of the spot and the decay of the spot is due to untwisting of the flux ropes. The main criticism of this model is the lack of observational evidence for large twist in sunspot magnetic fields or for untwisting during decay. Wilson stressed the similarities between the Parker and Meyer *et al.* models, rather than their differences. In Parker's model, the sunspot magnetic field divides into several smaller flux tubes a short distance below the solar surface. The Meyer *et al.* model is based on a single large flux tube, but Wilson suggested that this tube may be fragmented by internal convection and flux expulsion below a depth of about 2,000 km and hence looks very like the Parker model. Both models postulate a presently undetected sub-surface flow to provide stability for the sunspot, and this is perhaps their weakest feature.

Dynamical phenomena and fine structure in sunspots were reviewed, with R.L. Moore (NASA-Marshall Space Flight Center) presenting the observations and J.H. Thomas (University of Rochester) the theory. According to Moore, very high-resolution white-light photographs of sunspot penumbras show some of the dark fibrils to be elevated, translucent structures lying above a pattern of modified granulation. Thomas demonstrated how this might lead to a better understanding of the Evershed motion as a siphon flow (first proposed by F. Meyer and H.U. Schmidt in 1968) along elevated dark fibrils.

Moore showed that the observed umbral velocity oscillations in the photosphere and chromosphere and umbral flashes in the Ca II K and H $\alpha$  lines, with periods of about 3 min, are all manifestations of the same phenomenon, with the flashes corresponding to the strongest velocity oscillations. New observations reported at the meeting by B. Lites (Sacramento Peak Observatory), A.H. Nye (Rochester Institute of Technology) and F. Kneer (Kiepenheuer Institute) support this picture. Thomas showed how the umbral oscillations may be explained theoretically as a resonant mode of the entire sunspot atmosphere, excited by overstable convection in the subphotosphere.

The bright umbral dots are generally considered to be a small-scale convective phenomenon. E.N. Parker (University of Chicago) has shown that they may arise from penetration of hot, field-free gas from below due to overstability in the regions between flux tubes in his sunspot model. It was agreed that reliable observational data on the velocity, temperature and magnetic field in umbral dots were lacking.

Recent satellite measurements of dips in solar irradiance coincident with the passage of large sunspot groups across the solar disk (Willson *et al.* *Science* **211**; 700,

1981) have stimulated searches for effects of sunspots on the solar irradiance and luminosity, which were reviewed by P. Foukal (Atmospheric and Environmental Research, Inc.). H.S. Hudson (University of California, San Diego) presented a model of the effect of sunspots that accounts for the observed drops in solar irradiance. Observations suggest that the missing energy flux of sunspots is stored in the convection zone rather than being redistributed instantaneously over a large region of the solar surface. Theoretical calculations of the energy storage mechanism in the convection zone were presented by Foukal and by H. Spruit (Max Planck Institute for Astrophysics, Munich).

S. Vogt (Lick Observatory) reviewed the properties of spots on other stars. Work in the area has progressed rapidly to the point where certain classes of stellar light curve can be unambiguously interpreted as being caused by large dark spots on the surface of these stars. Direct measurement of magnetic fields associated with these spots is considerably more difficult, but recent studies by Vogt and his colleagues promise to yield reliable field strength measurements. Although strong similarities do exist between sunspots and

starspots, the long lifetime, large size and relatively hotter spot temperatures on the stars suggest important basic differences as well.

An announced goal of the workshop was to produce an overall empirical model of a typical sunspot atmosphere, analogous to the well known Bilderberg and Harvard-Smithsonian models of the quiet solar atmosphere. C. Zwaan (University of Utrecht) chaired the day-long session devoted to the model, which included important contributions concerning the umbral photosphere (P. Maltby, University of Oslo), chromosphere (H. Beebe, New Mexico State University; H.S. Yun, Seoul University; B. Lites, Sacramento Peak Observatory; and A. Skumanich, High Altitude Observatory) and transition region (K. Nicolas, Naval Research Laboratories). The participants agreed upon the properties of a one-dimensional empirical model of a sunspot atmosphere, and E. Avrett (Smithsonian Astrophysical Observatory) agreed to make the final calculations and prepare the model for publication in the conference proceedings. This model sunspot will be very useful to theoreticians and will not doubt serve as a focus for criticism and future improvements. □

### 100 years ago

At a recent meeting of the Banburyshire Natural History Society Mr. E. A. Walford read a note "On the Occurrence of a Fire-ball at Watergall" on August 23. In answer to Mr. Walford's queries, Mr. Fessey, jun., had sent an account as follows, dated "Watergall, Leamington, August 30: As regards the fire-ball, I was about 200 yards from it, in a waggon hovel. I saw it directly it left the sky, as I was looking in that direction at the time. When I first saw it, it looked like a ball of fire, about as large as a dinner-plate. It slowly descended, and I have no doubt I could have run twenty yards from the time I first saw it until it struck the ground; but when about fifteen to eighteen feet from the ground, it exploded with a loud crash, quite as loud as a cannon, distinctly before the thunder, which was very loud also. The explosion shook the whole buildings. I certainly thought the slates were falling in, but when it exploded one part struck the hedge, making a hole in the ground about a foot deep, and laying all the roots bare, but not damaging them. For some time the place looked all on fire, and there was a considerable quantity of smoke when it hit the ground, lasting for a second or two. It was seen by myself and four men. They also agree with me that this is as near as possible a correct explanation of it. We dug the hole out yesterday, but found nothing. The soil was blackened for several inches deep".

A severe earthquake was felt three weeks ago in the southern part of the North Island, New Zealand. No lives were lost, but in some of the townships in the Manawate district scarcely a chimney was left standing. In Foxton, for instance, no less than 250 were thrown down. Fissures extending for many miles are reported to have been made, and the railway line was

rendered unsafe in that neighbourhood, owing to the undulations of the earth alternately raising and depressing the rails. Since the large shock a good many of a slight nature have occurred. Two shocks of earthquake, each lasting from four to five seconds, were felt at noon on September 2 at Spalato in Dalmatia. The earthquake, which was accompanied by a subterranean rumbling, passed from the south-west to the north-east. It also made itself felt in the neighbouring islands of Brazza and Mascarsa, and in the town of Sebenico. A shock of earthquake was distinctly felt by several individuals at Courtown House, Gorey, Ireland, on August 27, at a quarter to five o'clock. Many heard a rumbling noise as thunder, some noticed the rattling of doors and windows, and one experienced what he called a "shiver". Lord Courtown noticed a rumbling noise, coming apparently from the north, passing under the house, and so away to the south; the door of the room in which he was sitting rattled. A slight shock of earthquake was felt at Naples at eight o'clock on Saturday morning. At about the same hour severer shocks took place at Popoli, Pescara, and Orsogna, in the Abruzzi. The seismographic instruments on Mount Vesuvius show great activity. At Atessa the church of St. Giustina was seriously damaged. There is a great panic everywhere amongst the population. A shock of earthquake occurred at Sanpietro Brazza (Dalmatia) on August 29, at 9 p.m. It lasted four seconds. Over forty shocks of earthquake have been felt at Khoi, Persia, between the 28th ult. and September 11. Some houses were destroyed, but no lives have been lost. Most of the inhabitants have left the town, and are encamped outside. The direction of the earthquakes was from north to south. The shocks were accompanied by rumbling noise.  
From *Nature* **24**, 476, 15 September, 1881.



## REVIEW ARTICLE

# Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds

Robert A. Lewis & K. Frank Austen

Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham, and Women's Hospital, Boston, Massachusetts 02115, USA

*The oxidative products of arachidonic acid and other mediators of immediate hypersensitivity are released by mast cells and other cells in response to the presence of noxious substances. In a normal response these chemicals produce the local inflammatory reactions that accompany destruction of the noxious substance. When the host response is misdirected or excessive, the concerted action of the same chemical mediators may produce allergic or inflammatory disease.*

ALLERGIC disorders of organs such as lung, nasal mucosa, skin and the gastrointestinal tract are due to a spectrum of pathobiological events which include vasodilation, increased vasopermeability, non-vascular smooth muscle contraction, leukocyte infiltration and local tissue destruction and repair. The allergic event is now known to be initiated by a stereospecific interaction of allergen and IgE antibody on the surface of the tissue mast cell; the pathobiological consequences are mediated by diverse substances that are released following immunological activation of the mast cell and are designated 'mediators of immediate hypersensitivity'.

The capacity to elaborate these mediators probably evolved as an adaptive response of the host to challenge by noxious external agents. The localization of mast cells at portals of entry into the host, the exquisite regulation of mediator generation and release, and the potency and site of each mediator action suggest a physiological role for the mediators in the regulation of the microenvironment<sup>1</sup>. The view that the mediators of inflammation are the 'hormones of the microenvironment' is consistent with their role in maintaining local homeostasis and with their capacity to produce clinical inflammation when the insult is excessive. Even the progression of the response to inflammation can be beneficial (host resistance) rather than detrimental (allergy) if, as a result, the noxious agent is eliminated. An important corollary is that disease arises not only when external forces overwhelm host resistance, but also when the host effectors are inappropriately and excessively recruited.

Because unstimulated mast cells do not contain all the biochemical mediators of IgE-provoked immediate hypersensitivity, some of them must either come from other cells or be newly generated during mast cell activation-secretion. The list of mediators of immediate hypersensitivity is growing but is, as yet, incomplete; for example, more is to be learned about the leukotriene (LT) components of slow reacting substance of anaphylaxis (SRS-A) which are biologically active oxidative metabolites of arachidonic acid; and we still have only a rudimentary understanding of the additional mediators released from other cells in response to the primary activation of mast cells by IgE.

## Cellular origins of prostaglandin D<sub>2</sub> and the leukotrienes

Release of arachidonic acid, the precursor of prostaglandins (PGs), by the action of phospholipase A<sub>2</sub> on mast cell membrane phosphatidylcholine (PC), occurs within 30 s of IgE-dependent activation<sup>2</sup>. In addition, selected membrane phospholipids are sequentially metabolized by phospholipase C and diglyceride lipase, with the resultant release of more arachidonic acid<sup>3,4</sup>.

Oxidative metabolism of arachidonic acid via the cyclooxygenase pathway sequentially generates two cyclic endoperoxides, PGG<sub>2</sub> and PGH<sub>2</sub> (refs 5, 6). PGH<sub>2</sub> is converted predominantly to PGD<sub>2</sub> by rat<sup>7,8</sup> and human<sup>8</sup> mast cells, presumably by the action of PGD<sub>2</sub> synthetase<sup>9</sup>. Purified rat mast cells, activated by the calcium ionophore A23187 or by reversed anaphylaxis, generate PGD<sub>2</sub> in quantities of 50.6 ± 34.4 and 8.0 ± 3.5 ng per 10<sup>6</sup> cells (mean ± s.d.), respectively. Human mast cells from lung parenchyma, dispersed by enzymatic digestion and purified to 70% by sequential gradient sedimentation, generate as much as 97 ng PGD<sub>2</sub> per 10<sup>6</sup> cells after activation by rabbit anti-human IgE<sup>8</sup>. Rat, but not human, mast cells also generate prostacyclin (PGI<sub>2</sub>) at about one-quarter the level of PGD<sub>2</sub>; relatively insignificant amounts of other oxidative metabolites of arachidonate are produced<sup>7,8</sup>. A major PGD<sub>2</sub> metabolite, 9 $\alpha$ -hydroxy,11,15-dioxo-2,3,4,5-tetranorprostan-1,20 dioic acid, has been isolated from the urine of patients with mastocytosis (this metabolite is barely detectable in normal human urine<sup>10</sup>), providing evidence that preferential synthesis of PGD<sub>2</sub> by human mast cells occurs *in vivo*. Because human lung fragments, challenged *in vitro* with an IgE-dependent stimulus, generate PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , PGI<sub>2</sub> and thromboxane A<sub>2</sub> (ref. 11), whereas the mast cell generates only PGD<sub>2</sub> (ref. 8), it is probable that the other mediators are generated by recruitment of secondary cell types.

This is also likely to be the case for the IgE-dependent generation of SRS-A in dispersed human lung cells<sup>12</sup>. It has long been known that this SRS-A can only be extracted after immunological challenge and is therefore not a preformed granule-associated mediator<sup>13</sup>. In addition, the IgE-dependent release of granule-associated histamine can be separated pharmacologically from the generation and release of SRS-A<sup>14-16</sup>; and the IgE-dependent release of SRS-A from either human lung fragments or enzymatically dispersed human pulmonary cells does not begin until histamine release is almost complete, whereas total SRS-A synthesis is biphasic and begins simultaneously with histamine release. These results suggest a two-compartment origin of SRS-A, possibly from different cells<sup>17</sup>. When enzymatically dispersed human pulmonary cells are separated on velocity gradients by the criterion of cell diameter, SRS-A generation by an IgE-dependent mechanism is limited to cell fractions containing mast cells, but decreases as the fractions become richer in mast cells. Whereas both human lung fragments and unfractionated pulmonary cells generated 40–100 units of SRS-A per  $\mu$ g of tissue or cellular histamine, cells sedimented by isopycnic and velocity gradients to maximize mast cell purity generate only 5–10 units of SRS-A per  $\mu$ g of cellular histamine. However, reconstitution of the partially

purified mast cell preparation with other gradient-processed pulmonary cells produces a threefold increase in the generation of SRS-A<sup>12</sup>. These studies imply that whereas the IgE-dependent generation of PGD<sub>2</sub> by human lung tissue is from mast cells alone, that of SRS-A leukotrienes requires the combined activation of mast cells and another cell population, probably pulmonary interstitial mononuclear cells.

### Chemical structure of the SRS-A leukotrienes

The biological activity now recognized as SRS-A may first have been observed 50 years ago in a study of spasmogenic activities from sputum of asthmatics<sup>18</sup>. Kellaway and Trethewie<sup>19</sup> subsequently showed that immediate-type hypersensitivity reactions generate a spasmogen which contracts smooth muscle more slowly than does histamine. This was confirmed by Brocklehurst<sup>20</sup> with the demonstration that H<sub>1</sub> antihistamine compounds failed to antagonize the spasmogenic activity of SRS-A. Mainly because of the small quantities available, even by the mid-1970s, all that was known chemically about SRS-A was that it was an acidic, highly polar lipid<sup>21,22</sup> of molecular weight (MW) < 700 (ref. 23), containing a sulphur atom in its structure<sup>24</sup>.

In 1977, Jakschik *et al.*<sup>25</sup> and Bach *et al.*<sup>26</sup> produced evidence that arachidonate was incorporated into SRS generated by rat basophilic leukaemia cells and rat peritoneal cells, respectively, following activation with A23187; however, incorporation did not follow the cyclooxygenase pathway<sup>27</sup>. Physicochemically and pharmacologically, this SRS was similar to SRS-A<sup>23,24</sup> generated in the rat by a pathway dependent on immune complexes, neutrophils and complement<sup>28,29</sup>. Morris, Piper and colleagues, studying the UV absorption spectrum, as had been done for partially purified SRS-A<sup>23</sup>, established that purified SRS-A from sensitized and antigen-challenged guinea pig lung had an UV absorption maximum ( $A_{\max}$ ) at 280 nm (ref. 30). Borgeat and Samuelsson<sup>31</sup> subsequently identified a metabolite of arachidonic acid, 5,12-di-hydroxyeicosatetraenoic acid, with an  $A_{\max}$  in the high-UV range, and recognized the relevance of its three conjugated double bonds to the previously noted<sup>23,30</sup> absorption spectrum of SRS-A. Murphy, Hammarström and Samuelsson<sup>32</sup> defined the parent SRS generated by ionophore activation of mouse mastocytoma cells as 5-hydroxy, 6-S-glutathionyl-7,9,11,14-eicosatetraenoic acid—leukotriene C (LTC). The specific structure of optically active centres and double-bond isomers was then defined and proved by total synthesis<sup>33</sup> to be 5(S)-hydroxy,6(R)-S-glutathionyl-7,9-trans,11,14,-cis eicosatetraenoic acid (LTC<sub>4</sub>).

Three groups of workers have described<sup>34–36</sup> an even more active spasmogen—the 6-sulphido-cysteinyglycine metabolite (LTD<sub>4</sub>)—apparently derived by the action of  $\gamma$ -glutamyl transferase on LTC<sub>4</sub>. Rat SRS-A<sup>28</sup> has subsequently been found to contain three leukotrienes—LTC<sub>4</sub>, LTD<sub>4</sub> and a 6-sulphido-cysteine metabolite (LTE<sub>4</sub>)<sup>37</sup>.

### Biological activities of leukotrienes and other newly generated mediators

Arachidonic acid oxidative metabolism is best viewed as a complex branching pathway (Fig. 1), the ultimate products and their biological effects being determined by which branch is followed. Different cell types may have different product profiles. For example, the activated mouse peritoneal macrophage generates comparable amounts of certain cyclooxygenase and 5-lipoxygenase products in the form of PGE<sub>2</sub>, PGI<sub>2</sub> and LTC<sub>4</sub> (ref. 38). In the stimulated human neutrophil, the 5-lipoxygenase pathway is preferentially activated to generate 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and 5-mono-hydroxyeicosatetraenoic acid (5-HETE), as well as 5,12-dihydroxyeicosatetraenoic acid (LTB<sub>4</sub>)<sup>31,39</sup>, whereas rat and human mast cells preferentially elaborate PGD<sub>2</sub> during coupled activation-secretion<sup>7,8</sup>.

The biological effects of the various products of the 5-lipoxygenase pathway vary considerably. 5-HETE is incorporated into membrane lipids<sup>40,41</sup>, modulating the motility<sup>39</sup> and possibly the

glucose transport<sup>42</sup> of neutrophils. LTB<sub>4</sub> is a potent chemotactic factor comparable with the fragments of the fifth component of complement<sup>43</sup>. LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> have vasoactive and spasmogenic activities which are more potent than those of histamine<sup>37</sup>. Thus, the processing of arachidonic acid via the 5-lipoxygenase pathway has three major classes of biologically active products—5-HETE, LTB<sub>4</sub> and the leukotriene constituents of SRS-A—and is not a simple detoxification pathway.

SRS-A has contractile activity on airway smooth muscle. This was initially demonstrated with crude SRS-A and human bronchioles<sup>13</sup>, and suggested its possible relevance to bronchial asthma. A preferential action of partially purified SRS-A on guinea pig parenchymal strips as compared with tracheal spiral preparations<sup>44</sup> was confirmed with purified and synthetic leukotriene constituents of SRS-A<sup>34,37,45</sup>. Furthermore, compared with histamine, the potency of the leukotrienes as spasmogens for the parenchymal strips, which contain peripheral airway smooth muscle, was greater by 2 logs for LTC<sub>4</sub> ( $EC_{50} = 1 \times 10^{-8}$  M) and LTE<sub>4</sub> ( $EC_{50} = 3 \times 10^{-9}$  M) and by 3 logs for LTD<sub>4</sub> ( $EC_{50} = 1$  to  $6 \times 10^{-10}$  M)<sup>12</sup>. Furthermore, intravenous administration of partially purified SRS-A<sup>46</sup> and of synthetic leukotrienes<sup>45</sup> results in a marked fall in the dynamic compliance of peripheral airways in intact unanaesthetized guinea pigs, whereas only minor increments in resistance occur in the more central airways. The doses of LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> that produce a 50% fall in dynamic compliance are 7, 1 and 7  $\mu$ g per kg, respectively<sup>45</sup>, whereas the doses of histamine and PGF<sub>2 $\alpha$</sub>  required to produce the same effect are 8 and 1,000  $\mu$ g per kg, respectively<sup>46</sup>. Although histamine seems to be as potent as LTC<sub>4</sub> and LTE<sub>4</sub>, but not as potent as LTD<sub>4</sub>, its effect on compliance lacks the duration of the leukotriene effects and is accompanied by an even more marked effect on central airways resistance<sup>45,46</sup>.

LTD<sub>4</sub>, but not LTC<sub>4</sub>, also has a hypotensive effect in the intact guinea pig, with 500 ng per kg producing a 50% fall in mean arterial pressure<sup>45</sup>. LTD<sub>4</sub> and LTE<sub>4</sub> are each ~100 times more potent than histamine in initiating an increase in vasopermeability in guinea pig skin, having a threshold dose of 5 ng per site. The cutaneous vasoactive effects of LTC<sub>4</sub> at the same dose include constriction as well as augmentation of venular permeability<sup>37,45</sup>.

There are at least three types of interactive effect of the various oxidative metabolites of arachidonate: (1) antagonistic or supportive for biosynthesis of a specific product, (2) synergistic or antagonistic at the target cells, and (3) augmentative due

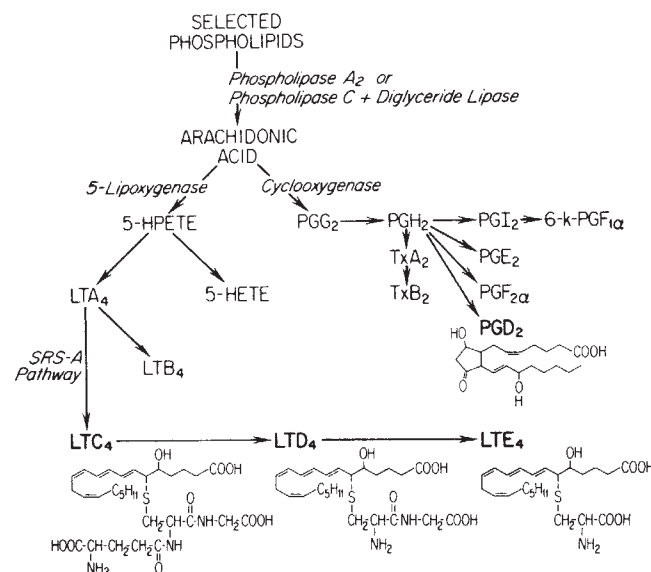


Fig. 1 Oxidative pathways of arachidonic acid metabolism, emphasizing mast cell-dependent products.



to the generation of secondary metabolites from cells activated by the initial product. Examples of interactions of the first type include the suppression and enhancement of SRS-A generation by human lung pretreated with  $\text{PGE}_1$  and  $\text{PGF}_{2\alpha}$ , respectively<sup>47</sup>, and the suppression of SRS-A generation in rat peritoneal cavity and guinea pig lung tissue pretreated with  $\text{PGI}_2$  (refs 48, 49). In the second type of interaction, at the level of the target cells,  $\text{PGD}_2$  acts synergistically on the chemotactic response of human neutrophils to  $\text{LTB}_4$  (ref. 50). The third type of interaction is exemplified by the elaboration of thromboxane by guinea pig airways contracting in response to the leukotriene constituents of SRS-A<sup>51</sup>, the thromboxane in its turn augmenting this response.

### Mast cell granule-associated mediators

The mast cell granule contains various enzymes, chemotactic peptides, heparin proteoglycan, histamine and, in rodents but not in humans, serotonin (Table 1)<sup>52</sup>. However, these account for less than half of the total estimated protein ( $56 \mu\text{g}$  per  $10^6$  cells) of rat serosal mast cell granules<sup>53</sup>, indicating that other activities have yet to be defined. In the human mast cell, the protein responsible for the structure of the secretory granule also remains to be elucidated<sup>54</sup>.

The function of heparin proteoglycan seems to be to store and possibly to concentrate other preformed mediators; solubilization of the heparin-mediator complex is necessary for their release. Rat heparin proteoglycan is a macromolecule of MW 750,000 (refs 55, 56), whereas human heparin proteoglycan is smaller<sup>57</sup>. The resistance of rat heparin proteoglycan to proteolytic cleavage is attributed to a core peptide of alternating glycine and serine residues in which two-thirds of the serine residues are substituted by heparin glycosaminoglycan chains<sup>56,58</sup>. Whereas only about one-third of heparin glycosaminoglycans of pig or human origin have anti-thrombin III binding and associated anticoagulant activities<sup>57</sup>, they all inhibit the alternative complement pathway<sup>59,60</sup>; this suggests that heparin released from mast cells activated by the anaphylatoxic cleavage fragment of the fifth component of complement<sup>61,62</sup> down-regulates the production of additional activating peptide.

The presence of acid hydrolases such as  $\beta$ -hexosaminidase,  $\beta$ -glucuronidase and arylsulphatase in rat<sup>63,64</sup> and human<sup>65</sup> mast cell secretory granules (Table 1) suggests that these granules are modified primary lysosomes<sup>52</sup>. Rat mast cells also contain a small number of non-secretory lysosomes which differ from the secretory granules in containing arylsulphatase B but not A, as well as an acid phosphatase<sup>63,66,67</sup>. The pH optimum of the acid hydrolases is broad enough to be compatible with some extracellular function. The neutral proteases of the mast cell granule, which in the rat are chymotryptic<sup>68,69</sup> and possibly related to cathepsin G (ref. 70), and in the human are tryptic<sup>65</sup>, may have extracellular action on proteoglycan core peptides, collagen and elastin. The enzymatic content of the secretory granules may thus have a role in clearing the products of damaged tissue, and thus facilitating its repair.

Eosinophils accumulate in tissues undergoing mast cell-mediated immediate hypersensitivity reactions, and the term eosinophil chemotactic factor of anaphylaxis (ECF-A)<sup>71,72</sup> is used to describe the activity generated during such reactions in human or guinea pig lung slices. Approximately half of the crude activity has been resolved into two tetrapeptides<sup>73</sup> which are not only chemotactic for eosinophils *in vitro*<sup>73</sup> and *in vivo*<sup>74</sup>, but which can also increase the number of functional eosinophil receptors for the major cleavage fragment of the third component of complement<sup>75</sup>, C3b. It remains to be directly proved that the human mast cell is the source of ECF-A.

In patients with physical allergy, in whom an experimental stimulus produces angioedema/urticaria and tissue mast cell degranulation, the plasma becomes enriched with histamine, a family of acidic eosinophilotactic factors ranging in size from the tetrapeptides to polypeptides, and a high-molecular-weight chemotactic factor for neutrophils<sup>76-78</sup>. Because this last factor has also been observed in the plasma of asthmatic patients

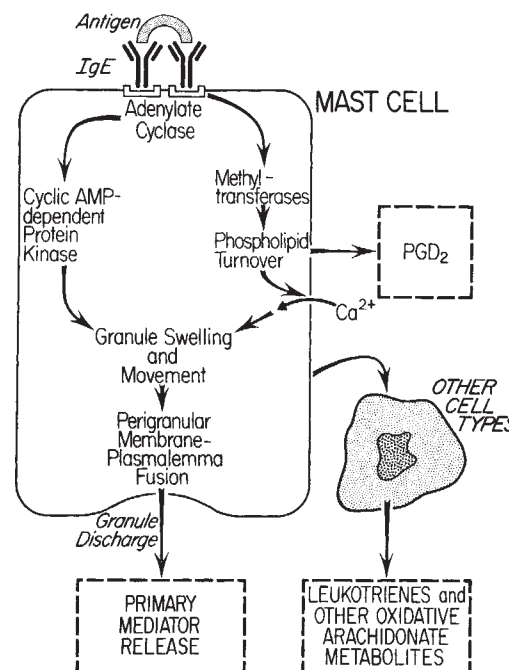


Fig. 2 Pathway of mast cell activation-secretion to primary mediator release and arachidonic acid metabolism.

undergoing aerosol allergen-induced bronchospasm<sup>79,80</sup> but not in patients with a similar pathological pulmonary response to isocapnic hyperventilation<sup>80</sup> or noxious chemicals<sup>81</sup>, it is thought to be generated by the immunological activation of mast cells. These factors, whether primary mast cell granule constituents or secondarily elaborated products from another source, presumably facilitate host resistance at mucosal and cutaneous surfaces of the mast cell-rich organs.

### The activation-secretion response

The stereospecific perturbation of the mast cell IgE receptor initiates a transmembrane signal which results in the solubilization and movement of the swollen secretory granules, the fusion of the perigranular membrane with the plasma membrane and the extracellular release of the granule constituents (Fig. 2). The IgE membrane receptor of rat basophil leukaemia cells is composed of an  $\alpha$ -subunit of MW 50,000 and a  $\beta$ -subunit of MW 30,000. Radioiodination of intact cells indicates that only a portion of the  $\alpha$ -subunit is exposed to the extracellular medium, and it is this domain, termed  $\alpha_2$ , which binds IgE. The remainder of the  $\alpha$ -subunit ( $\alpha_1$ ) and the  $\beta$ -subunit are intramembranous<sup>82,83</sup>. Dimerization of IgE Fc receptors, presumably through an effect on a transmembrane coupling protein, results in transmembrane activation of a discrete adenylate cyclase subpopulation which generates cyclic AMP from ATP. The cellular concentration of cyclic AMP peaks within 5–15 s of receptor perturbation<sup>84,85</sup> and activates cytoplasmic cyclic AMP-dependent protein kinase holoenzymes<sup>86</sup>. Adenosine and adenosine analogues modified in the purine portion of the molecule ('R-site' active)<sup>87,88</sup> act via the membrane on the coupling unit of the adenylate cyclase complex, causing it to increase in activity and raise the cellular levels of cyclic AMP without inducing mediator release. R-site agonists interact synergistically with the IgE-receptor perturbation to raise cytoplasmic levels of cyclic AMP and to increase the rate and quantity of mediator release in a dose-related fashion<sup>89</sup>. Conversely, adenosine analogues modified in their ribose portion (P-site active) act on the P site of the adenylate cyclase molecule to inhibit enzymatic activity<sup>87,88</sup>, attenuate the rise in cellular cyclic AMP associated with IgE-receptor perturbation, diminish the activation of cytoplasmic cyclic AMP-dependent protein kinase and suppress mediator release<sup>89</sup>. Thus, the coupled

activation-secretion response to stereospecific perturbation of the IgE Fc receptors includes the sequential transmembrane activation of associated adenylate cyclase, elevation of cytoplasmic levels of cyclic AMP ('second messenger') and activation of cyclic AMP-dependent protein kinase.

Another set of early biochemical events that follow receptor perturbation and are essential to an interactive and effective secretory response have recently been suggested. The sequential actions of two methyltransferases catalyse the methylation of membrane phosphatidylethanolamine to form phosphatidylcholine, with concomitant activation of calcium ion channels and increased calcium ion flux<sup>90,91</sup>. Inhibition of this phospholipid methylation step by dibutyl cyclic AMP suppresses mediator release but not the activation of adenylate cyclase<sup>2,91</sup>, whereas blockade of the active site of the adenylate cyclase molecule by a ribose-modified adenosine analogue suppresses mediator release but not phospholipid methylation<sup>89</sup>. As suppression of either membrane adenylate cyclase or methyltransferase inhibits IgE-dependent activation-secretion, it is apparent that these are parallel but not sequential early events. The recent finding<sup>2</sup> that the introduction of diisopropylfluorophosphate (DFP) prevents not only mediator release<sup>92</sup> but also the methylation of phospholipid and the rise in cellular levels of cyclic AMP suggests that the uncovering of a membrane serine protease may precede the other early membrane-related biochemical changes.

The dose-related inhibition of mediator release by pretreatment of cells with methylxanthine is associated with a rise in cellular levels of cyclic AMP and activation of both type I and type II cyclic AMP-dependent protein kinases<sup>93,94</sup>. Although this inhibition could reflect the phosphorylation and thus inactivation of a protein critical in the activation-secretion sequence, an alternative possibility is that it reflects competitive depletion of cyclic AMP-dependent holoenzymes<sup>95</sup>. By analogy with endocrine cells and platelets<sup>96,97</sup>, the precondition for the uptake of water and the granule swelling that occur before granule discharge may be an increase in granule membrane ion permeability brought about by cyclic AMP-dependent phosphorylation of a perigranular membrane protein. Because they are essential for the ATP- and actinomyosin-dependent contraction of microfilaments<sup>98</sup>, calcium ions probably have a direct role in the movement of secretory granules to the mast cell surface for discharge. The combined effects of granule swelling and contraction of cytoskeletal elements could then bring the perigranular membrane into contact with the plasmalemma. IgE-dependent activation-secretion of concentrated human mast cells is characterized by solubilization of the crystalline secretory granules within the first minute, and by the ordering of intermediate filaments around the swollen granule followed by fusion of the perigranule membrane with the membranes of other granules or with the plasmalemma<sup>54</sup>. Parallel generation of plasma membrane lysophospholipids and diacylglycerol by the actions of calcium-dependent phospholipases could facilitate membrane fusion<sup>3,99</sup> and exposure of the contents of the mast cell granule to the extracellular fluid environment.

### Implications for physiological and pathobiological responses in humans

The preliminary data available on human cellular responses to the leukotrienes *in vitro* and *in vivo* indicate that minimal concentrations of these agonists produce effects characteristic of a host inflammatory response. On human bronchi, LTC<sub>4</sub> and LTD<sub>4</sub> are spasmogens, more than 1,000 times as potent as histamine and 500 times more potent than PGF<sub>2α</sub> on a molar basis<sup>100</sup>. LTC<sub>4</sub> and LTD<sub>4</sub> elicit a wheal and flare in the human at 0.1–1.0 nmol per site<sup>50</sup>, compared with a 1–10 pmol range for histamine<sup>101</sup>, but the flare caused by the leukotrienes is much more persistent, suggesting some effects on arterioles or deeper venous elements<sup>50</sup>. LTB<sub>4</sub> has a measurable chemotactic effect on human neutrophils *in vitro* at a concentration of 3 × 10<sup>-9</sup> M, which is a 10–100-fold lower threshold than that for 5-HETE<sup>43</sup>

**Table 1** Mediators of rat and human mast cell secretory granules

|                   | Rat                          |       | Human                        |
|-------------------|------------------------------|-------|------------------------------|
|                   | mast cells × 10 <sup>6</sup> | μg    | mast cells × 10 <sup>6</sup> |
|                   |                              | Units | Units                        |
| Protein (total)   | 56                           |       |                              |
| β-hexosaminidase  | 0.22                         | 1.1   | 3.4                          |
| β-glucuronidase   | 0.11                         | 0.16  | 0.03                         |
| Arylsulphatase    | <0.1                         | 0.09  | 0.03                         |
| Chymase           | 24                           | 0.6   | <0.05                        |
| Tryptase          |                              | <0.02 | 0.9                          |
| Histamine         | 12                           |       | 1.7                          |
| Serotonin         | 1                            |       | 0                            |
| Heparin           |                              |       |                              |
| glycosaminoglycan | 30                           |       | 4.0                          |

Units are defined according to the capacity of each enzyme to cleave measured quantities of its synthetic substrate in standard conditions<sup>64,65</sup>

and is quite comparable with that for C5a or C5a<sub>des Arg</sub> (ref. 102). *In vitro*, LTB<sub>4</sub> is more chemotactic for human eosinophils<sup>103</sup>, in terms of both potency and activity, than are the eosinophilatonic tetrapeptides<sup>104</sup>. In human skin, LTB<sub>4</sub> is chemotactic for neutrophils at doses of only 0.3–1.6 nmol (ref. 50); no comparative data are available for other chemotactic factors.

A combination of the lipoxygenase and cyclooxygenase products of arachidonate metabolism can have a synergistic pro-inflammatory action. Thus, the cutaneous infiltrate elicited by the injection of LTB<sub>4</sub> into the skin of rabbits, monkeys and humans is augmented by the presence of vasodilating prostaglandins such as PGE<sub>2</sub> and PGD<sub>2</sub> (refs 50, 105). In the human, PGD<sub>2</sub> alone elicits a transient wheal and flare, whereas LTB<sub>4</sub> produces a time-dependent indurated and tender lesion with a peak effect after several hours. When a subclinical dose of LTB<sub>4</sub> is given with PGD<sub>2</sub>, the local wheal and flare subsides only to be followed by the appearance of an indurated lesion, which biopsy reveals to be rich in neutrophils<sup>50</sup>. These interactive effects may be of great clinical importance in that the inhibition of the cyclooxygenase pathway by a non-steroidal anti-inflammatory agent, with attenuated generation of cyclooxygenase products and augmented production of lipoxygenase products, could be beneficial or detrimental depending on the responsiveness of target tissues and cells to the altered product profile. Furthermore, the recent findings that C5a-induced contraction of guinea pig ileum and C5a/C5a<sub>des Arg</sub>-induced increased venular permeability involve recruitment of oxidative products of arachidonate<sup>106,107</sup> suggest that the physiologically equivalent situation may require combination of the leukotrienes with cyclooxygenase products.

Although the mast cell primary granule-associated mediators manifest vasoactive, permeability-augmenting, spasmogenic and chemotactic activities which can modulate the micro-environment so as to recruit protective proteins and cells to the inflammatory site, it may be that the secondary mediators are critical to a fully developed local host response. The interaction of the oxidative products of arachidonate metabolism with each other and with the mediators of the mast cell secretory granule may provide the intensity of local response needed for subclinical, homeostatic regulation of the microenvironment at cutaneous and mucosal surfaces and around venules which are constantly exposed to noxious substances. The recruitment of the oxidative products of arachidonate from both motile and tissue-fixed secondary cell types affords an amplification mechanism for these host responses. Just as the alternative complement pathway serves to distinguish self from non-self without the exquisite specificity of adaptive immunity, and can, in addition, augment an immunologically specific complement response<sup>1,108</sup>, so the interactive functions of the chemical mediators derived from arachidonic acid can be brought into action by both immunological and non-immunological stimuli to amplify the effects of other mediators.



The leukotriene constituents of SRS-A can, for example, be generated by immune complex-, complement- and polymorphonuclear leukocyte-dependent mechanisms in the rat<sup>28</sup>. This may be relevant to the synovial fluid abnormalities of patients with active rheumatoid arthritis, where the presence of immune complexes<sup>109</sup> undergoing complement-dependent phagocytosis by neutrophils<sup>110</sup> is associated with the generation of the chemotactically active LTB<sub>4</sub> (ref. 111). It is likely that LTB<sub>4</sub> is generated by the neutrophils themselves and serves to amplify the profound neutrophilia characteristic of rheumatoid effusions. Furthermore, it is possible that non-steroidal anti-inflammatory agents fail to reduce remissions because, although they inhibit arachidonate metabolism, they may augment the synthesis of the lipoxigenase products.

The leukotriene constituents of SRS-A may prove to be important mediators of the reversible loss of pulmonary elasticity and the ventilation-perfusion inequalities with hypoxia that are observed in asthmatics even when central airways are sufficiently patent to minimize wheezing<sup>112,113</sup>. The effects of aerosol administration of partially purified SRS-A to monkeys are predominantly on peripheral, rather than central, airways<sup>114</sup>, as was observed previously in studies in intact guinea pigs<sup>46</sup>. Both monkey lung and human lung sensitized *in vitro* with serum of ragweed-sensitive humans release SRS-A leukotrienes on challenge with antigen or anti-IgE<sup>34,115,116</sup>. In sensitized rhesus monkeys, the acetylenic analogue of arachidonic acid, 5,8,11,14-eicosatetraynoic acid (ETYA), a competitive inhibitor of all oxidative pathways of arachidonate metabolism,

prevents the bronchoconstriction induced by an IgE- and mast cell-dependent response to aerosolized antigen<sup>117</sup>. As this response is also partially blocked by an end-organ antagonist of SRS-A leukotriene function, FPL-55712, but not by the cyclooxygenase inhibitor indomethacin<sup>118</sup>, the 5-lipoxygenase pathway may be preferentially involved. Furthermore, as the adverse reactions to non-steroidal anti-inflammatory agents, characterized by wheezing, urticaria and angioedema, are not immunological, the mechanism may involve a shift in arachidonate metabolism to the lipoxigenase pathway.

The speculations that the leukotrienes are involved in the pathobiology of immune complex disease and of allergic and non-allergic bronchial asthma, imply that pharmacological intervention must aim to reduce the oxidative metabolism of arachidonic acid via the 5-lipoxygenase pathway. Because steroid hormones suppress the activation of cellular phospholipases<sup>119</sup> and hence the elaboration of arachidonic acid, it is possible that a major anti-inflammatory action of steroids involves substrate limitation of arachidonic acid metabolism via both pathways. Non-steroidal inhibitors of arachidonate metabolism by both pathways suppress antigen-induced bronchospasm in monkeys<sup>117</sup> and zymosan-induced cutaneous inflammation in guinea pigs<sup>107</sup>. Although these are encouraging findings, the agents are not suitable for clinical investigation and the animal models are too limited to be relevant to human disease.

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## ARTICLES

# Planetary-scale wave guides in the troposphere and stratosphere

W. A. Chapman\* & T. Miles†

\* Department of Physics, Heriot-Watt University, Edinburgh EH14 4AS, UK

† Department of Meteorology, University of Edinburgh, Edinburgh EH9 3JZ, UK

*Standing eddy fluxes of geopotential are derived from Northern Hemisphere conventional and satellite data. Vertical and meridional wave guide behaviour is clearly evident for planetary-scale wave disturbances in the extratropical winter troposphere and stratosphere. In accordance with zonal harmonic perturbation model studies (steady-state and linearized) of forced planetary wave propagation, the observed standing wave geopotential flux patterns are characterized by a marked dependence on zonal wavenumber and the basic state transmission properties (refractive index squared). Transports for the middle and upper stratosphere are based on satellite measurements of thermal radiation and represent the first quantitative analysis at these levels of two-dimensional wave guide effects associated with individual zonal wave disturbances for a quiescent stratospheric winter period.*

THE Asian High and Icelandic Low are noteworthy examples of wave-like deviations from zonal (longitudinal) symmetry present on seasonal circumpolar charts of sea-level pressure. Hemispheric analysis of conventional (radiosonde) and satellite (radiance) data has shown that such standing wave disturbances extend into the stratosphere and mesosphere during winter, and are confined to tropospheric levels in summer<sup>1–4</sup>. Steady-state numerical results obtained from both global general circulation and linearized mechanistic models have established that the atmospheric standing eddies are primarily forced in the troposphere through the combined influence of large-scale orography and net diabatic heating processes<sup>5–9</sup>.

Observational and model investigations of the seasonal balance of kinetic energy have revealed that the stratospheric winter circulation is strongly influenced by tropospheric planetary-scale wave disturbances through a vertical transfer of wave mechanical energy at the tropopause 'boundary'<sup>5,6,10–14</sup>. This external dynamical forcing arises through the rate of work exerted vertically (per unit area of the boundary surface) by the existing pressure perturbation forced in the troposphere. At a given latitude and height, the pressure-work surface effect therefore involves a correlation between pressure and vertical velocity. However, when pressure is used as the 'vertical' coordinate, the same effect is given by the correlation between the geopotential height of the pressure surface and  $\omega$  the 'pressure-velocity'. This may be regarded as a flux of geopotential through an isobaric surface.

The pressure-work forcing mechanism has also been interpreted—from both a theoretical<sup>15</sup> and observational<sup>16</sup> viewpoint—as representing vertical wave propagation or a vertical wave energy flux. Perhaps of more relevance to the present study is the apparently close relationship between wave propagation and the wave pressure-work (geopotential flux) effect discussed in linearized and steady-state model investigations of forced tropospheric standing wave disturbances<sup>7,17–23</sup>. These models predict the occurrence of vertical (and meridional) wave guides in the troposphere and stratosphere which are critically dependent on the zonal wavelength of the forced disturbance and the transmission properties (refractive index squared) of the zonally averaged atmosphere.

Previous observational studies of stratospheric energy transports using satellite radiance data have analysed net eddy mechanical energy flux contributions, both for a Southern Hemisphere winter period<sup>24</sup> and on a daily basis during a Northern Hemisphere major stratospheric sudden warming event<sup>25</sup>. The principal aim of the present investigation, however, is to evaluate two-dimensional wave guide characteristics (on a seasonal basis) of individual standing zonal wave disturbances in the Northern Hemisphere troposphere and stratosphere for a relatively undisturbed stratospheric winter period. This type of analysis permits comparison of conventional and satellite analyses and also facilitates the interpretation of the observed wave guide features in terms of steady-state and linearized model results for the Northern Hemisphere.



## Observational technique

The present study uses two basic sources of hemispheric data: (1) conventional objective analyses of temperature ( $T$ ) and geopotential height ( $H$ ) for 13 standard constant pressure levels from 850 to 10 mbar ( $\sim 30$  km) processed daily for 12.00 GMT by the National Meteorological Center, Washington (NMC)<sup>26,27</sup>, and (2) daily-mean latitude-longitude radiance analyses derived by the Atmospheric Physics Department, Oxford University, through interpolation of declouded day-night orbital measurements of IR radiation from the selective chopper radiometer (SCR) on Nimbus 5 (refs 28, 29). NMC and SCR analyses were subsequently averaged for the period 1 December 1973–13 February 1974, NMC fields interpolated from a polar stereographic to a latitude-longitude format and then zonal harmonic analysis applied to NMC and SCR time-mean grids (resolution:  $4^\circ$  lat.  $\times 10^\circ$  long.). A statistical 'minimum information' retrieval formulation<sup>30</sup>, appropriate for 'point' radiance measurements, was modified through Fourier series expansion<sup>31</sup> to permit retrieval between  $20$  and  $80^\circ$ N of zonal harmonic temperature coefficients from winter-mean zonal harmonic radiance coefficients for the eight SCR  $\text{CO}_2$  sounding channels. The retrieved temperature coefficients, on 35 constant in pressure levels, were then used to compute the SCR geopotential height coefficients through hydrostatic integration from an NMC reference height analysis level of 300 mbar. Data for the period following 13 February were not included in the present 'quiet' winter analysis because of the onset of a major stratospheric sudden warming and zonal-mean wind reversal<sup>32,33</sup>.

From these time-mean fields, latitude-pressure cross-sections were computed for the zonal geostrophic wind ( $[\bar{u}]$ ), and the eddy flux of geopotential ( $\bar{z} = g\bar{H}$ ) by zonal wavenumbers 1 and 2 (W1, W2), where  $g$  is gravitational acceleration (constant) and time- and zonally averaged quantities are denoted by an overbar and square bracket, respectively. Standing eddy flux of geopotential (FOG) components were evaluated from expressions derived by Eliassen and Palm<sup>34</sup> for steady-state, geostrophic, frictionless and adiabatic flow

$$[\bar{\rho}][(\bar{v})^*(\bar{z})^*] = -[\bar{\rho}][\bar{u}][(\bar{u})^*(\bar{v})^*] \quad (1)$$

$$\frac{1}{g}[(\bar{\omega})^*(\bar{z})^*] = -\frac{f[\bar{u}]}{g[\bar{S}]}[(\bar{v})^*(\bar{T})^*] \quad (2)$$

where for a given pressure ( $p$ ) and latitude ( $\phi$ ) the local deviation from the zonal mean is denoted by  $(\bar{v})^*$ ,  $\rho$  is density,  $f$  the Coriolis parameter,  $2\Omega \sin \phi$ , with  $\Omega$  the Earth's angular rotation speed,  $v$  the meridional velocity, and in isobaric coordinates, adiabatic vertical velocity is  $\omega$  and static stability,

$$S = -(p/p_0)^{R/c_p} \frac{\partial \theta}{\partial p} \quad (3)$$

with  $\theta$  potential temperature,  $p_0$  a reference pressure (1,000 mbar),  $R$  the dry air gas constant, and  $c_p$  the specific heat of air at constant pressure. Equations (1) and (2) indicate that for westerly basic state flow an upward and equatorward FOG is associated with a poleward geostrophic flux of heat and momentum—or equivalently, a westward and eastward standing wave phase displacement with increasing height and latitude, respectively<sup>34</sup>. Equations (1) and (2) were expanded (using the geostrophic approximation for  $u$  and  $v$ ) in terms of zonal Fourier series coefficients in spherical polar coordinates for evaluation on constant pressure surfaces. Linear interpolation with respect to  $\ln p$  was used to calculate NMC static stabilities, with the 10-mbar values derived in part from both climatology and SCR retrieved temperatures for 5 mbar (refs 13, 16).

Representative values of the zonally averaged refractive index squared for the 1973–74 winter were calculated from the gridded  $[\bar{u}]$  and  $[\bar{T}]$  fields by adopting the formulation derived by Matsuno<sup>19</sup> in a linearized numerical investigation of steady-state, geostrophic, two-dimensional forced wave propagation in spherical coordinates. For the case of standing waves superim-

posed on an adiabatic and frictionless flow, the index of refraction squared,  $\bar{N}$ , becomes

$$\bar{N}(K) = [\bar{u}]^{-1} \left\{ 2\Omega a \cos \phi + [\bar{u}] \sec^2 \phi - \frac{\partial^2 [\bar{u}]}{\partial \phi^2} + \frac{\partial [\bar{u}]}{\partial \phi} \tan \phi - \frac{fa}{[\bar{T}]} \frac{\partial}{\partial p} \left( \frac{[\bar{T}]}{[\bar{S}]} \frac{\partial [\bar{T}]}{\partial \phi} \right) \right\} - \frac{(fa)^2}{4pR[\bar{S}]} - (K \sec \phi)^2 \quad (4)$$

where  $a$  is the Earth's mean radius, zonal wavenumber  $K \equiv 2\pi a \cos \phi / \text{zonal wavelength}$ , and from the zonal thermal wind relation, the vertical shear of the basic state flow

$$\frac{\partial [\bar{u}]}{\partial \ln p} = \frac{R}{fa} \frac{\partial [\bar{T}]}{\partial \phi} \quad (5)$$

The zonally averaged transmission properties are therefore represented here through the combined effects of the vertical component of the Earth's vorticity and spherical geometry,  $[\bar{u}]$  magnitude, sign, shear and curvature, compressibility, static stability and zonal wavenumber. Vertical centred finite differences were used to determine both  $[\bar{S}]$  and term 5 in equation (4).

## Observed basic state properties and standing wave fluxes

Figure 1a shows the NMC and SCR  $[\bar{u}]$  cross-sections for the regions  $20$ – $80^\circ$ N,  $850$ – $10$  mbar and  $300$ – $\sim 0.3$  mbar, respectively. In terms of the main westerly jet features, the 1973–74 winter zonal wind distribution is broadly similar to that previously derived from midwinter climatological studies incorporating conventional data<sup>13,16,35</sup>. Winter-mean values of  $\bar{N}(0)$  for 1973–74 are presented in Fig. 1a and indicate that local contributions arising from  $[\bar{u}]$  shear and curvature significantly influence index variations. For example, in the upper troposphere and lower stratosphere, the sign of  $\bar{N}(0)$  reverses north of  $\sim 30^\circ$ N. As shown in Table 1, the negative (positive) values near  $40^\circ(50$ – $70^\circ)$ N are associated with positive (negative) meridional curvature in the presence of easterly (westerly) vertical shear (note that the effect of meridional shear is negligible). Index values in the upper stratosphere increase south of  $40^\circ$ N within a region of decreasing westerly  $[\bar{u}]$  and vertical shear.

The corresponding geopotential height amplitude and FOG cross-sections for the W1 and W2 disturbances are shown in Fig. 1b and c, respectively. A vector representation of the FOG components has been adopted which incorporates scaling dependent only on height; consequently, the plotted FOG vectors at a given height are directly comparable over latitude and between wavenumbers. At a given point in the latitude-pressure domain, a vector represents the resultant standing eddy FOG (zonally averaged) contribution per unit area for an individual wavenumber component. In terms of the local energy balance for the zonally averaged atmosphere, the two-dimensional divergence of the FOG components represents an 'external' source of eddy kinetic energy<sup>10</sup>. (Note, however, that an alternative approach to the diagnosis of planetary wave propagation for the zonally averaged atmosphere has been discussed by Edmon *et al.*<sup>36</sup>.) The analysed geopotential height wave amplitudes are shown for completeness; they do not necessarily directly depend only on the eddy FOG divergence, because the time-mean amplitude structure may also be influenced by *in situ* baroclinic, barotropic, dissipative and nonlinear processes.

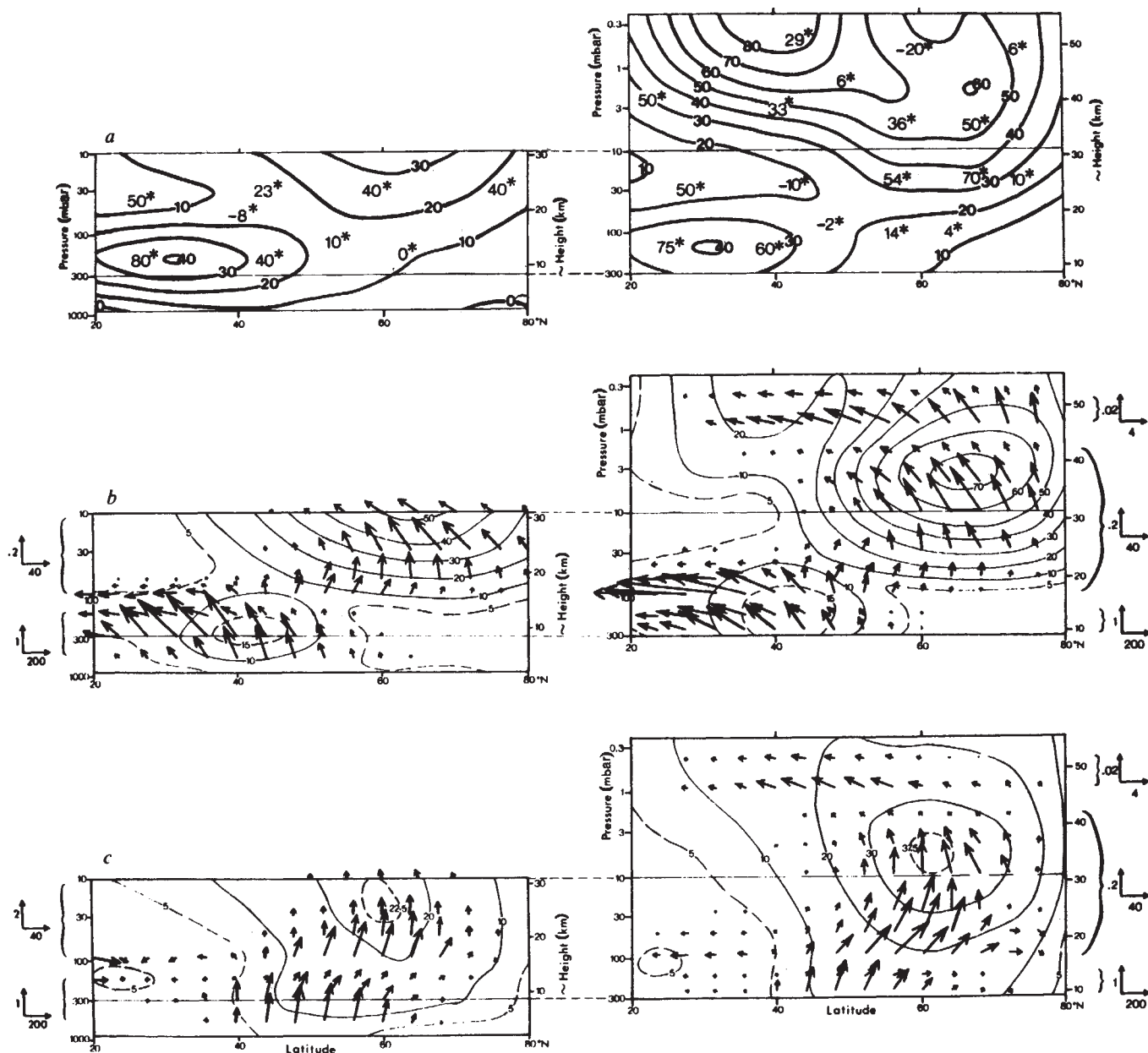
In the troposphere, maximum W1 amplitudes and an upward FOG are observed at  $\sim 40^\circ$ N. At upper troposphere and lower stratosphere levels, the vertical flux divides into an equatorward and poleward branch in the region of negative  $\bar{N}(0)$ , this effect being prominent in both NMC and SCR analyses. The nature of the observed FOG bifurcation and  $\bar{N}(0)$  variations implies that the two FOG branches may represent subtropical and polar wave guides. The relative importance of zonal wavenumber dependence for  $K = 1$  (see equation (4) and Table 2) increases in polar latitudes with the  $\bar{N}(0)$  values, shown in Fig. 1a, further

reduced for W1. At the tropopause level, the polar W1 FOG branch tunnels through a relatively shallow negative  $\bar{N}(1)$  layer, guided towards the polar jet core axis and region of positive  $\bar{N}(1)$ .

A noteworthy feature of the subtropical W1 guide in the upper troposphere is the suggestion of a meridional FOG convergence south of the basic state jet core. This wave guide may therefore represent a possible source of kinetic energy for eddy and perhaps basic state components of the tropical circulation. The W1 height disturbance in the lower polar stratosphere is centred slightly north of the polar jet core axis and characterized by a divergent upward FOG, whereas in the middle and upper stratosphere an increasing meridional FOG transfer occurs. The stratospheric vector patterns suggest that W1 vertical FOG convergence is principally confined to polar latitudes at levels above  $\sim 10$  mbar with divergence below. As in the troposphere, an equatorward W1 guide is present in the upper stratosphere with the FOG vectors apparently refracted from negative  $\bar{N}(1)$

values to positive. The W1 meridional FOG is a maximum at  $\sim 40$  km, with a flux convergence south of  $50^\circ\text{N}$  as indicated by tabulated values (not shown). Compared with lower levels, the W1 amplitude structure in the upper stratosphere is characterized by an increased meridional scale, similar to that observed for the zonal wind field.

The W2 amplitude and FOG patterns in Fig. 1c differ from those for W1 in several important aspects. First, the W2 disturbance is centred between  $40$  and  $70^\circ\text{N}$  throughout the troposphere and stratosphere, although some increase in meridional scale occurs above  $\sim 25$  km. The tropospheric component is located more directly below the polar jet core axis, and is associated with a marked vertical FOG concentrated along this jet axis with relatively small meridional transfers towards tropical and polar latitudes. Second, in terms of the vertical FOG, both NMC and SCR analyses indicate that the W2 vertical penetration is less than that for W1, particularly if vertical flux contributions are averaged with respect to latitude. The



**Fig. 1** Latitude-pressure cross-sections for the Northern Hemisphere 1973-74 winter, showing conventional (left) and satellite-derived (right) zonally averaged distributions of: *a*, zonal (west-east) geostrophic wind in  $\text{m s}^{-1}$  and selected values of the basic state refractive index squared  $\bar{N}(0)$  (denoted by asterisk), and standing zonal wavenumber 1 (*b*) and 2 (*c*) geopotential height amplitudes in dm and associated flux of geopotential vectors in  $\text{W m}^{-2}$ . Meridional and vertical vector components are everywhere scaled in the ratio 200:1, thereby preserving vector direction; vector magnitudes, however, vary with height for the layers indicated. Further details of the vector representation are given in the text.



**Table 1** Zonally averaged refractive index squared  $\bar{N}(K)$  for conventional and satellite analyses for the 1973–74 winter

| Latitude–pressure<br>grid coordinates | NMC              |                  | SCR              |                  | 40°N,<br>3 mbar |
|---------------------------------------|------------------|------------------|------------------|------------------|-----------------|
|                                       | 40°N,<br>61 mbar | 60°N,<br>33 mbar | 44°N,<br>27 mbar | 56°N,<br>27 mbar |                 |
| Terms: 1 + 2                          | 49               | 22               | 37               | 20               | 16              |
| 3                                     | –11              | 14               | –29              | 27               | 10              |
| 4                                     | 1                | 0                | 1                | 2                | 1               |
| 5                                     | –43              | 11               | –14              | 13               | 10              |
| 6                                     | –4               | –7               | –5               | –8               | –4              |
| $\bar{N}(0)$                          | –8               | 40               | –10              | 54               | 33              |
| $\bar{N}(1)$                          | –10              | 36               | –12              | 51               | 31              |
| $\bar{N}(2)$                          | –15              | 24               | –18              | 41               | 26              |

Values are presented for selected points in Fig. 1a for  $K = 0, 1$  and 2. Also included are contributions arising from terms 1–6 in equation (4) (those independent of  $K$ ).

observed difference in vertical structure is consistent with theory through the increased wavenumber (squared) dependence in equation (4) in polar latitudes (see Table 2). Third, in the subtropical upper stratosphere, a meridional FOG convergence is observed for the W2 component, although the magnitude of the flux is generally less than 50% of that observed for the W1 disturbance at this level. (Uncertainties in the objectively analysed height fields are known to be responsible for the distorted NMC W2 meridional FOG pattern in the upper troposphere between 20 and 25°N (ref. 37).)

## Discussion

The magnitude and direction of the FOG vectors observed for the 1973–74 winter are in broad agreement with results from previous time–mean standing eddy flux investigations for the Northern Hemisphere troposphere and lower-middle stratosphere<sup>11–14,36,38–42</sup>, particularly if allowances are made for the influence of interannual variability, uncertainties associated with the basic conventional hemispheric analyses<sup>43</sup> (see below) and differences in the evaluation of eddy transports (for example, the inclusion of transient eddy FOG contributions<sup>12,14,38</sup>, or the use of a constant static stability for the vertical FOG component<sup>40,42</sup>). The pattern of equatorward FOG vectors described here for the middle-upper stratosphere supports the wave guide interpretation proposed by Barnett<sup>44</sup> through harmonic analysis of the Northern Hemisphere stationary W1 radiance component during 1973–74 for the SCR channel B12 centred at ~45 km.

The NMC and SCR vector patterns are characteristic of zonal wave guide effects described in steady-state perturbation model studies of one- and two-dimensional propagation of Northern Hemisphere planetary-scale wave disturbances forced in the troposphere<sup>7,17,23</sup>. Aspects of the model solutions featured in the 1973–74 winter analysis include: (1) zonal wavenumber dependence on vertical penetration, and ‘tunnelling’ of standing wave mechanical energy through a shallow minimum  $\bar{N}(K)$  barrier in the tropopause region, as first proposed by Charney and Drazin<sup>17</sup>, (2) ducting or concentration of wave mechanical energy along the stratospheric polar jet core axis<sup>19,20</sup> and towards tropical latitudes in the upper troposphere<sup>19</sup> and middle-upper stratosphere<sup>18,19,22</sup>, (3) variations in meridional scale between lower and upper stratospheric wave disturbances<sup>22</sup> and (4) reduced vertical penetration for the polar W2 disturbance<sup>19,20</sup>. The Matsuno<sup>19</sup> and Simmons<sup>20</sup> model solutions predict differences in the depth of W2 penetration of ~10–15 km along the polar jet core axis in the lower and middle stratosphere. The SCR W2 geopotential height amplitude (Fig. 1c) is a maximum along the polar jet axis at ~35 km, 60°N, and closely resembles the analytical solution obtained by Simmons<sup>20</sup> for a two-dimensional basic state zonal flow similar to that depicted in Fig. 1a for the region 15–45 km and 40–80°N. However, we hesitate to draw firm conclusions regarding detailed comparisons of vertical penetration in view of the probable uncertainties in the SCR analyses (see below).

**Table 2** Contribution by term 7 ( $-K^2 \sec^2 \phi$ ) in equation (4) to the zonally averaged refractive index squared  $\bar{N}(K)$ 

| Latitude $\phi$    | 20–30° | 40° | 50° | 60° | 70° | 80°  |
|--------------------|--------|-----|-----|-----|-----|------|
| Zonal wavenumber 1 | –1     | –2  | –2  | –4  | –9  | –33  |
| Zonal wavenumber 2 | –5     | –7  | –10 | –16 | –34 | –133 |

Close examination of Fig. 1 and Table 1 reveal differences between the SCR and NMC 1973–74 seasonal analyses. Such observational ‘errors’ are not unexpected for several reasons: (1) the inherent vertical separation and width of the SCR channel weighting functions results in only poor resolution of features with a vertical scale less than ~5 km, (2) zonal wave coefficients and corresponding transports may be distorted by vertical compensations which arise during the temperature retrieval process, and (3) comparison of the NMC 30-mbar seasonal diagnostics with those derived from independent hemispheric analyses of the same conventional radiosonde reports (see Table 3) has revealed discrepancies >25%. The differences evident in Table 3 probably reflect sampling errors in the initial data sets and first-guess ‘virtual reports’. The magnitude of uncertainty associated with the ‘ground-truth’ diagnostics should therefore be borne in mind when judging the usefulness of the SCR seasonal analyses in the stratosphere.

The influence of variations in wavenumber retrieval procedures on the SCR flux patterns will be discussed elsewhere. Standing eddy heat and momentum transports, flux contributions for other seasons and higher zonal wavenumbers,  $\bar{N}(K)$  cross-sections, the accuracy of conventional seasonal stratospheric height and temperature analyses and comparison with dynamical models will also be examined.

## Conclusions

The observational evidence presented here indicates that dynamical interaction between the extratropical troposphere and quiescent stratosphere for the Northern Hemisphere winter is characterized by vertical and meridional propagation of standing planetary-scale waves primarily forced in the troposphere. The nature of propagation implied from the FOG vector patterns suggests a marked dependence both on zonal wavenumber and the two-dimensional transmission properties (refractive index squared) of the basic state. Such a wave guide interpretation accords with results from steady-state linearized model investigations, and seems to be closely related to the question of the role of standing eddies in determining the winter-mean balance of kinetic energy in the subtropical and polar stratosphere.

The present study reveals considerable agreement between NMC and SCR seasonal flux analyses despite substantial intrinsic differences between conventional and satellite observing systems. A noteworthy feature is the observed wavenumber-dependent FOG bifurcation in the upper troposphere and ducting of wave mechanical energy in the stratospheric polar jet

**Table 3** Comparison of planetary-scale diagnostics derived from independent conventional winter-mean hemispheric analyses of temperature and geopotential height (NMC, Washington, and Berlin University)\*

| 1973–74 winter, 30 mbar, 60°N                            |    | NMC  | Berlin |
|----------------------------------------------------------|----|------|--------|
| $[\bar{u}]$ ( $\text{m s}^{-1}$ )                        |    | 26.3 | 29.7   |
| Geopotential height                                      | W1 | 33.8 | 37.2   |
| Amplitude (dm)                                           | W2 | 23.2 | 32.0   |
| $[(\bar{v})^*(\bar{T})^*]$ ( $\text{m s}^{-1}\text{K}$ ) | W1 | 16.5 | 14.3   |
|                                                          | W2 | 9.6  | 14.2   |
| $[(\bar{u})^*(\bar{v})^*]$ ( $\text{m}^2\text{s}^{-2}$ ) | W1 | 17.2 | 2.1    |
|                                                          | W2 | 1.1  | 10.9   |

Quantities displayed are the basic state zonal wind, and for zonal wavenumbers 1 and 2, the standing wave amplitude and meridional flux of heat and momentum.

\* The same radiation corrections are applied and satellite thicknesses excluded.

region as predicted by Matsuno<sup>19</sup> and Simmons<sup>20</sup>. Statistical retrieval of satellite radiance measurements has permitted an extension of the conventional FOG analysis into the upper stratosphere, thereby allowing the first quantitative observational evaluation of seasonal, two-dimensional wave guide effects above the 10-mbar level for a relatively undisturbed Northern Hemisphere winter. Perhaps the most striking result of the SCR 1973–74 flux analysis in the upper stratosphere is the marked equatorward FOG by both W1 and W2 disturbances. The broad agreement between SCR and NMC analyses and wave propagation theory provides further evidence to support

the view that interpretation of satellite retrieved temperature analyses, on a global and seasonal basis, provides valuable insight into the dynamical behaviour of stratospheric planetary-scale wave disturbances and interaction with the troposphere.

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# Formation of a 2'-phosphomonoester, 3',5'-phosphodiester linkage by a novel RNA ligase in wheat germ

Maria Konarska\*, Witold Filipowicz\*, Horst Domdey<sup>†‡</sup> & Hans J. Gross<sup>†</sup>

\* Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 36 Rakowiecka str., 02-532 Warsaw, Poland

† Institut für Biochemie, Roentgenring 11, D-8700 Würzburg, FRG

*Extracts of wheat germ contain an RNA ligase activity that catalyses the conversion of linear polyribonucleotides into covalently closed circles. The newly formed 3',5'-phosphodiester bridge is resistant to alkali and a number of ribonucleases. This unusual stability is due to the presence of a phosphoryl group esterified to the 2'-position of the 3'-nucleotide joined. This is the first demonstration of an RNA ligase in higher plants and of a natural 2'-phosphomonoester, 3',5'-phosphodiester linkage.*

MATURATION of eukaryotic RNAs frequently involves the excision of intron sequences and subsequent ligation of RNA segments<sup>1</sup>. The enzymatic mechanism responsible for this unique processing is of considerable interest. At present two enzyme activities are known to possess RNA ligase activity: one, purified from *Escherichia coli* infected with phage T4, catalyses the intra- and intermolecular joining of 5'-phosphate and 3'-hydroxy termini<sup>2</sup>. The second, described more recently<sup>3–5</sup>, catalyses the joining of the two halves of tRNA molecules after excision of the intron from tRNA precursors in *Saccharomyces cerevisiae* and *Xenopus* oocytes. In contrast to the T4 RNA ligase, these enzymes require 5'-hydroxy and 3'-phosphate termini. Apart from these activities, isolated nuclei of adenovirus-infected HeLa cells process viral mRNA precursor molecules, that is excise intron sequences and ligate RNA fragments<sup>6</sup>. A similar enzyme system that performs post-transcriptionally both the endonucleolytic cleavage and ligation of an intron-containing precursor of ribosomal RNA has been found in nuclei<sup>7</sup> and nucleoli<sup>8</sup> of *Tetrahymena thermophila*.

Here we show that extracts of wheat germ contain an RNA ligase activity able to convert linear polyribonucleotides into

covalently closed circular molecules. This activity uses as a substrate 5'-hydroxy-terminated RNA molecules bearing a phosphate at the 3' terminus. Preliminary evidence indicates that a 2',3'-cyclic phosphate rather than a 3'-phosphomonoester is required. Surprisingly, the newly formed 3',5'-phosphodiester linkage is insensitive to many ribonucleases and to alkali. We provide evidence that this resistance is due to a phosphomonoester group in the 2' position at the site of the 3',5'-phosphodiester linkage.

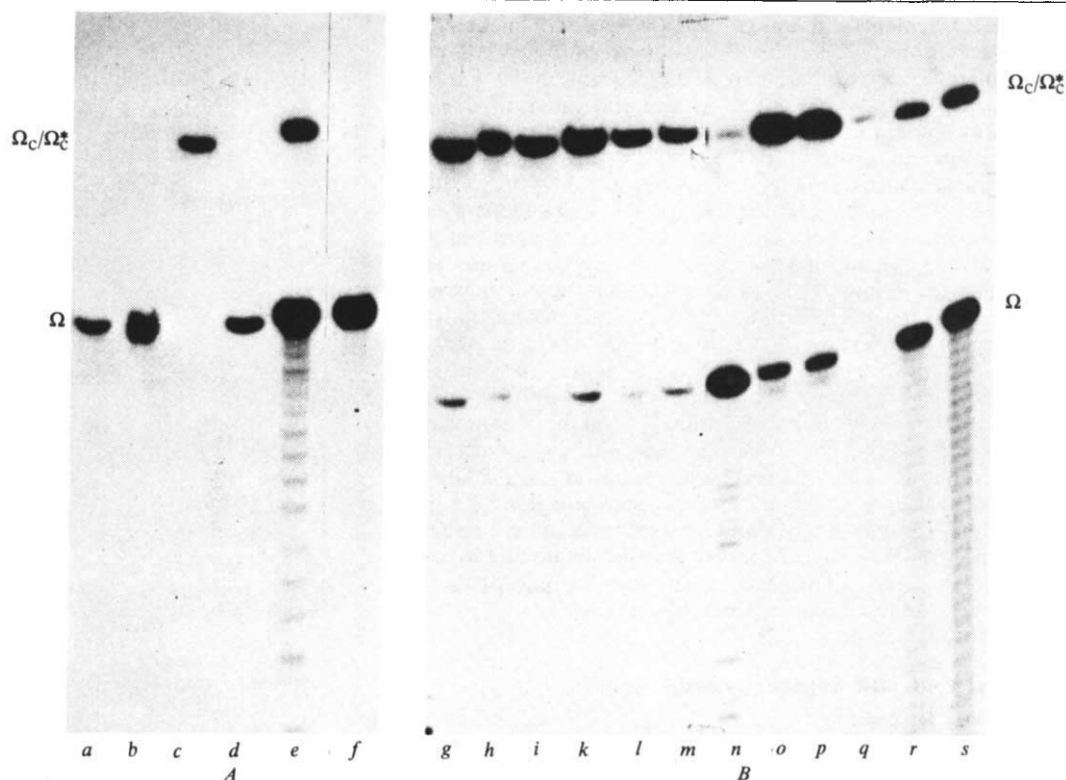
## Conversion of a TMV RNA fragment to a covalently closed circle by wheat-germ extracts

The polyribonucleotide used throughout is the RNase T<sub>1</sub>-resistant leader fragment (73 nucleotides long) of tobacco mosaic virus (TMV) RNA (SPS isolate)<sup>9</sup>. The fragment was prepared by treating the viral RNA with RNase T<sub>1</sub> and phosphatase, and was

‡ Present address: Institut Suisse de Recherches Experimentales sur le Cancer, CH-1066 Epalinges sur Lausanne, Switzerland.



**Fig. 1** Conversion of linear  $\Omega$  fragments to circular molecules in wheat-germ extract. **A**, unlabelled leader fragment ( $\Omega$ ) of TMV (SPS isolate) RNA was isolated, phosphatase treated and labelled at the 5' end as described elsewhere<sup>9</sup>. 5'-OH-, 3'-<sup>32</sup>P-labelled  $\Omega$  was prepared either by RNase T<sub>1</sub> cleavage of  $\Omega$  circles ( $\Omega_c$ ) which had been obtained by incubation of T4 RNA ligase with 5'-<sup>32</sup>P-labelled  $\Omega$  or by T4 RNA ligase-catalysed addition of <sup>32</sup>P-pCp to the 3' end of  $\Omega$  (ref. 9) and subsequent treatment with RNase T<sub>1</sub>. Incubation was carried out in 5  $\mu$ l containing: 20 mM HEPES-KOH, pH 7.4, 4 mM MgAc<sub>2</sub>, 100 mM KAc, 80  $\mu$ M spermine, 10 mM creatine phosphate, 60  $\mu$ g ml<sup>-1</sup> of creatine kinase, 2.5 mM ATP, 10% dimethyl sulphoxide (DMSO), 1  $\mu$ l of 23,000g supernatant (~50  $\mu$ g protein) prepared from commercial wheat germ<sup>10</sup> and 5–10  $\times 10^3$  c.p.m. of appropriate <sup>32</sup>P-labelled  $\Omega$  fragment. After incubation at 30 °C for 7 min, samples were made up to 8 M with urea, electrophoresed on 20% polyacrylamide-8M urea gel

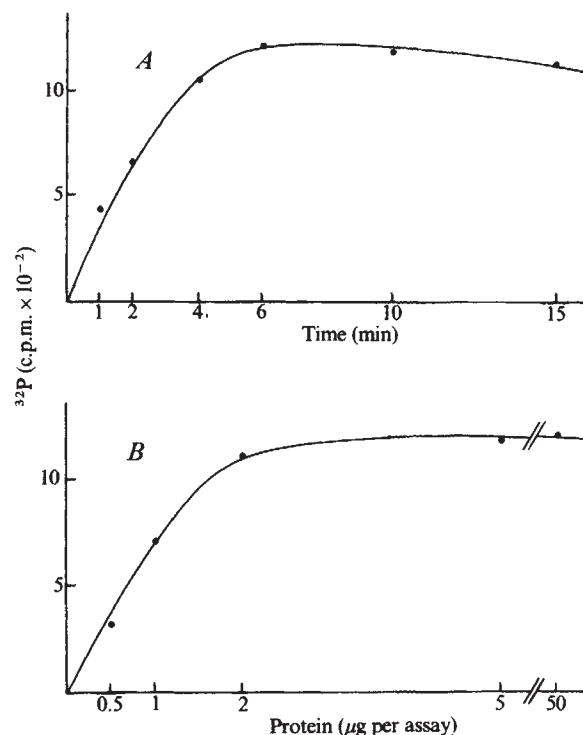


and autoradiographed as described elsewhere<sup>9</sup>. **a**, [5'-<sup>32</sup>P] $\Omega$ ; **b**, [5'-<sup>32</sup>P] $\Omega$ ; **c**, [3'-<sup>32</sup>P] $\Omega$  incubated with wheat-germ extract; **d**, [3'-<sup>32</sup>P] $\Omega$  incubated with wheat-germ extract; **e**, [3'-<sup>32</sup>P] $\Omega$  incubated with wheat-germ extract; **f**, [3'-<sup>32</sup>P] $\Omega$  incubated as in **e** except that ATP was omitted from the reaction mixture. **B**, circular  $\Omega$  formed in wheat germ ( $\Omega_c$ ) was excised and eluted from the gel in the presence of tRNA carrier.  $\Omega_c$  or  $\Omega_c^*$  (200–500 c.p.m.) was treated with different enzymes: polynucleotide phosphorylase<sup>13</sup> (**g**,  $\Omega_c$ ; **h**,  $\Omega_c^*$ ); tobacco acid pyrophosphatase (1.7 U, 60 mM NaAc, pH 5.0, 10 mM mercaptoethanol, 1.2 mM Na<sub>2</sub>EDTA, for 1 h at 37 °C) (**i**,  $\Omega_c$ ; **k**,  $\Omega_c^*$ ); untreated material (**l**,  $\Omega_c$ ; **m**,  $\Omega_c^*$ ); RNase T<sub>1</sub> (1 U, 50 mM Tris-HCl, pH 7.8 for 1 h at 37 °C) (**n**,  $\Omega_c$ ; **o**,  $\Omega_c^*$ ); calf intestine phosphatase (20 mU, 50 mM Tris-HCl, pH 8.0 for 1 h at 37 °C) (**p**,  $\Omega_c$ ; **q**,  $\Omega_c^*$ ); 50 mM NaOH (2 min at 80 °C) (**r**,  $\Omega_c$ ; **s**,  $\Omega_c^*$ ). Samples were analysed by electrophoresis and autoradiography as in **A**.

then purified by electrophoresis on a polyacrylamide gel. This fragment, called  $\Omega$  (ref. 9), can be conveniently labelled with <sup>32</sup>P at its 5' end using [ $\gamma$ -<sup>32</sup>P]ATP and polynucleotide kinase from T4 phage-infected *E. coli*. Such [5'-<sup>32</sup>P] $\Omega$  molecules are readily converted into circles by T4 RNA ligase<sup>9</sup>.  $\Omega$  fragments bearing a <sup>32</sup>P label at the 3' end ([3'-<sup>32</sup>P] $\Omega$ ) can be obtained by RNase T<sub>1</sub> cleavage of the purified <sup>32</sup>P-labelled circular molecules ( $\Omega_c$ ) from above, or by ligation of 5'-[<sup>32</sup>P]pCp to the 3' end of  $\Omega$ , followed by removal of the terminal Cp by treatment with RNase T<sub>1</sub> (ref. 9).

Incubation of [3'-<sup>32</sup>P] $\Omega$  with a post-mitochondrial (S23) or post-ribosomal supernatant (S100) prepared from commercial wheat germ<sup>10</sup> resulted in the formation of RNA molecules that migrated in 20% polyacrylamide-8 M urea gels like authentic  $\Omega$  circles (Fig. 1). Incubation of [5'-<sup>32</sup>P] $\Omega$  with wheat-germ extract in identical conditions did not lead to the appearance of this new RNA band (Fig. 1A). When the wheat-germ extract was first incubated for 10 min at 57 °C or pretreated with proteinase K, the new form of  $\Omega$  was not generated from [3'-<sup>32</sup>P] $\Omega$  (data not shown), indicating that a protein is involved in its formation. In appropriate conditions the yield of product is proportional to incubation time and protein concentration (Fig. 2). ATP and Mg<sup>2+</sup> are absolute requirements for the formation of the new  $\Omega$  species from [3'-<sup>32</sup>P] $\Omega$ ; however, the non-hydrolysable analogues  $\alpha$ ,  $\beta$ -methylene-ATP and  $\beta$ , $\gamma$ -methylene-ATP did not substitute for ATP in this reaction (data not shown).

Evidence that the product formed from [3'-<sup>32</sup>P] $\Omega$  on incubation with wheat-germ extracts corresponds to a covalently closed form of  $\Omega$  is presented in Fig. 1B. When this material was purified by electrophoresis and subjected to mild alkali treatment (lane **s**), the predominant product was a molecule having the size of linear  $\Omega$ , in addition to further degradation products of the latter. This agrees with the expected single nick conversion of circular RNA into its linear counterpart<sup>9,11,12</sup>. The same typical degradation pattern was observed for circular  $\Omega$  molecules synthesized by T4 RNA ligase (Fig. 1 and ref. 9). In



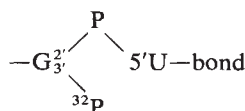
**Fig. 2** Conversion of linear  $\Omega$  fragments into circles by wheat-germ ligase; dependence on time and protein concentration.  $10^4$  c.p.m. of [3'-<sup>32</sup>P] $\Omega$  were incubated with wheat-germ 23,000g supernatant and the products analysed by gel electrophoresis as described in Fig. 1 legend. Circularized  $\Omega$  ( $\Omega_c^*$ ) was cut out and its radioactivity determined. **A**, dependence of  $\Omega_c^*$  formation on time of incubation (protein concentration was 50  $\mu$ g per 5  $\mu$ l assay as for Fig. 1). **B**, dependence of  $\Omega_c^*$  formation on protein concentration in a 5- $\mu$ l assay. Incubation time was 7 min.

either case, fragments with a mobility between that of linear and circular forms of  $\Omega$  were not observed. Such fragments of intermediate size would result from the degradation of linear dimer  $\Omega$  molecules or from  $\Omega$  molecules ligated to some component in the wheat-germ extract.

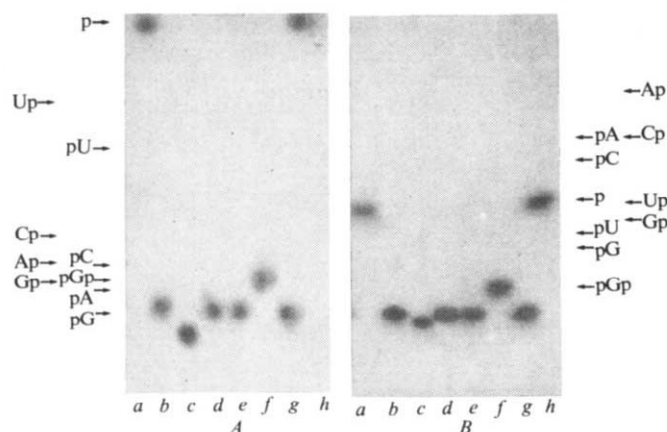
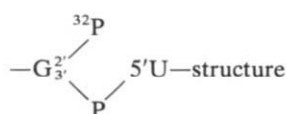
To characterize further the circular  $\Omega$  synthesized in wheat-germ extracts (called hereafter  $\Omega_c^*$ , in contrast to  $\Omega_c$ , the circle synthesized by commercial T4 RNA ligase) it was treated with several enzymes. Parallel digestions of  $\Omega_c$  were performed. As expected,  $^{32}\text{P}$ -labelled  $\Omega_c$  and  $^{32}\text{P}$ - $\Omega_c^*$  were susceptible to hydrolysis with RNase T<sub>2</sub>, nuclease P<sub>1</sub>, RNase A (not shown) and resistant to 3'-exonucleolytic degradation with polynucleotide phosphorylase in the presence of inorganic phosphate<sup>13</sup>. As reported previously, RNase T<sub>1</sub> generates linear  $\Omega$  molecules from  $\Omega_c$  due to the susceptibility of the 3',5'-phosphodiester bond between the 3'-terminal G and the 5'-terminal U (lane *n*, Fig. 1*B* and ref. 9). Most unexpectedly, however,  $\Omega_c^*$  was resistant to RNase T<sub>1</sub> (lane *o*), and, moreover, the  $^{32}\text{P}$  label in  $\Omega_c^*$  was sensitive to calf intestine phosphatase (lane *q*), in contrast to  $\Omega_c$  (lane *p*). Both  $\Omega_c$  and  $\Omega_c^*$  were resistant to tobacco acid pyrophosphatase, which cleaves polyphosphate and pyrophosphate linkages<sup>14</sup>. The latter result and the phosphatase susceptibility of the  $^{32}\text{P}$  label exclude a ligation of  $\Omega_c^*$  by a pyro- or polyphosphate bond.

### Structure of the bond formed by the novel ligase

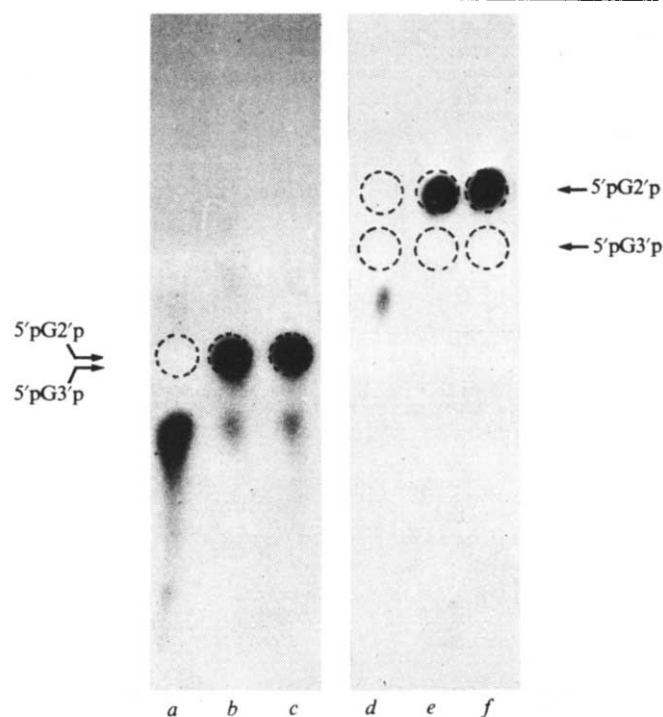
The RNase T<sub>1</sub> resistance of  $\Omega_c^*$  could be due to methylation of the single G to m<sup>7</sup>G or Gm, but this would not explain the phosphatase sensitivity of  $\Omega_c^*$ . Two structures, however, would explain RNase T<sub>1</sub> resistance of  $\Omega_c^*$  and phosphatase sensitivity of the  $^{32}\text{P}$  label: either a 2',5'-phosphodiester bond between the 3'-terminal  $^{32}\text{P}$ -Gp and the 5'-terminal U, that is, a



or a normal 3'-5' bond with the original 3'- $^{32}\text{P}$  label translocated to the 2' position, that is a



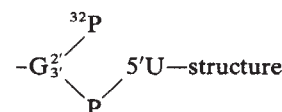
**Fig. 3** Analysis of the products of enzymatic and alkaline digestion of  $\Omega_c^*$ .  $\Omega_c^*$  was treated in 5  $\mu\text{l}$  with: *a*, calf intestine phosphatase (conditions as in Fig. 1*B*); *b*, RNase T<sub>2</sub> (2 U, 10 mM NaAc, pH 4.5, 1 mM Na<sub>2</sub>EDTA, for 3 h at 37 °C); *c*, nuclease P<sub>1</sub> (1  $\mu\text{g}$ , 50 mM NH<sub>4</sub>Ac, pH 5.3, for 1 h at 50 °C); *d*, 50 mM NaOH (0.5 h at 90 °C); *e*, RNase T<sub>2</sub> followed by spleen phosphodiesterase (1  $\mu\text{g}$ , 50 mM Na-citrate, pH 6.5 for 1 h at 37 °C); *f*, nuclease P<sub>1</sub> followed by snake venom phosphodiesterase (1  $\mu\text{g}$ , 50 mM Tris-HCl, pH 7.8 for 1 h at 37 °C); *g*, RNase T<sub>2</sub> followed by snake venom phosphodiesterase; and *h*, RNase T<sub>2</sub> followed by calf intestine phosphatase. TLC on cellulose plates was performed in *t*-butanol/HCl/H<sub>2</sub>O (14:3:3) (*A*), or isobutyric acid/conc. NH<sub>3</sub>/H<sub>2</sub>O, pH 4.3 (577:38:385) (*B*). Positions of appropriate nucleotide markers are indicated.



**Fig. 4** The labelled dinucleotide obtained from  $\Omega_c^*$  after nuclease P<sub>1</sub> treatment releases  $[2'\text{-}^{32}\text{P}]\text{p5'G2'p}$  on further digestion with snake venom phosphodiesterase. The structure of  $\text{p5'G2'p}$  was confirmed by (1) resistance of the  $^{32}\text{P}$ -2' label to the 3' phosphatase activity of nuclease P<sub>1</sub> (ref. 14) and (2) its chromatographic co-migration with authentic  $\text{p5'G2'p}$ .  $\Omega_c^*$  was digested with: *a, d*, nuclease P<sub>1</sub>; *b, e*, nuclease P<sub>1</sub> followed by snake venom phosphodiesterase; and *c, f*, nuclease P<sub>1</sub>, followed by snake venom phosphodiesterase and again nuclease P<sub>1</sub>. Samples were analysed on TLC cellulose plates in two different solvents: *a-c*, isobutyric acid/conc. NH<sub>3</sub>/H<sub>2</sub>O, pH 4.3 (577:38:385); or *d-f*, saturated ammonium sulphate/1 M NaAc/isopropanol (80:18:2)<sup>30</sup>. A mixture of authentic  $\text{p5'G2'p}$  and  $\text{p5'G3'p}$  was prepared by acid treatment of  $\text{p5'G3'p}$  (1 M HCl, 1 h at 37 °C)<sup>31</sup>. The resistance of our  $\text{p5'G2'p}$  marker to nuclease P<sub>1</sub> and snake venom phosphodiesterase was confirmed in independent experiments. Positions of nucleotide markers are indicated.

A third and most unlikely structure, containing a phosphorylated guanine base, can be excluded because of the alkali resistance of the newly formed bond. Degradation products of  $^{32}\text{P}$ - $\Omega_c^*$  were therefore analysed by TLC as shown in Fig. 3. Extensive digestion with nuclease P<sub>1</sub>, RNase T<sub>2</sub> or alkali did not yield any of the four labelled ribonucleoside monophosphates, but the labelled material behaved like a dinucleoside diphosphate or triphosphate. The fact that the products of both nuclease P<sub>1</sub> and RNase T<sub>2</sub> digestion were radioactive clearly indicated that the label could not be located in the terminal 5' or 3' position of the product. Treatment of  $^{32}\text{P}$ - $\Omega_c^*$  with nuclease P<sub>1</sub>, followed by snake venom phosphodiesterase, yielded a product with chromatographic properties of  $\text{p5'G2'p}$  or  $\text{p5'G3'p}$ . This indicates the existence in  $\Omega_c^*$  of a phosphodiester linkage that is blocked by an additional 2'- or 3'-phosphomonoester group. The nuclease P<sub>1</sub> resistance also supports the proposed structures, as the action of this enzyme is known to be impaired by 2'-O-methylation<sup>15</sup>.

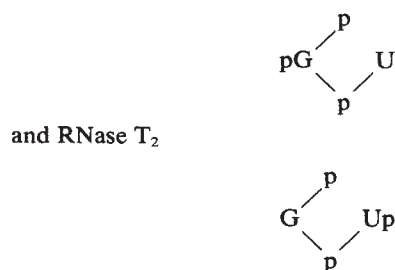
Conclusive evidence for a



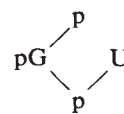
is given in Fig. 4. The nucleoside diphosphate generated from  $^{32}\text{P}$ - $\Omega_c^*$  by the consecutive treatment with nuclease P<sub>1</sub> and snake venom phosphodiesterase migrates together with authentic  $\text{p5'G2'p}$  and not with  $\text{p5'G3'p}$ . Moreover, the 2'- $^{32}\text{P}$  label is resistant to the 3'-phosphatase activity of nuclease P<sub>1</sub> (ref. 16), which easily dephosphorylates  $\text{p5'G3'p}$ . As expected from results obtained with 2'-O-acetylated polynucleotides<sup>17</sup>, the



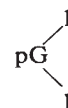
RNase T<sub>2</sub>-resistant dinucleotide from <sup>32</sup>P-Ω<sub>c</sub>\* is not degraded further by spleen phosphodiesterase (Fig. 3). These findings are fully supported by the experiments shown in Fig. 5. The dinucleotides resistant to nuclease P<sub>1</sub>



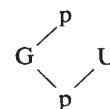
both have five negative charges. Periodate oxidation and β-elimination of the 3'-terminal nucleoside<sup>18</sup> of



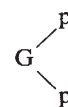
yields a compound with six negative charges



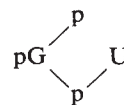
that is, 2',3',5'-GTP, whereas similar treatment of the dinucleotide resistant to both RNase T<sub>2</sub> and nuclease P<sub>1</sub>



three negative charges, produces a nucleoside diphosphate



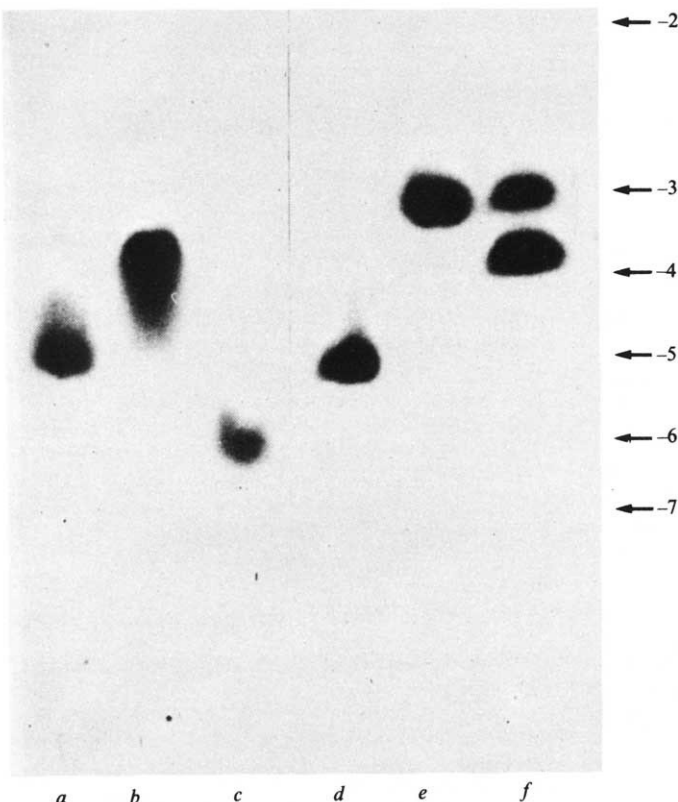
four negative charges. So far we have provided direct evidence only for the 3'-terminal G of linear 3'-<sup>32</sup>P-Ω participating in the newly formed bond, and not for the presence of the 5'-terminal U residue. Sequence analysis of the nuclease P<sub>1</sub>-resistant dinucleotide from <sup>32</sup>P-Ω<sub>c</sub>\* is shown in Fig. 6. The mobility shift<sup>19</sup> caused by partial snake venom phosphodiesterase digestion of



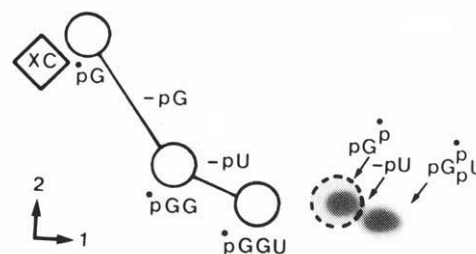
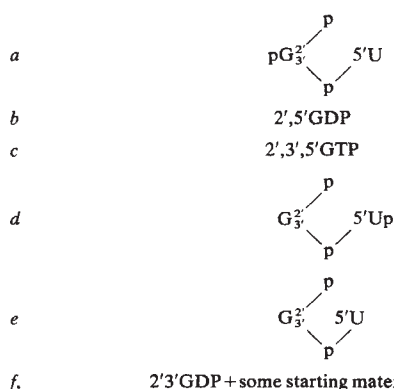
is typical for the removal of pU, whereas the resulting <sup>32</sup>P-p5'G2'p co-migrates with authentic p5'G3'p.

## Conclusions

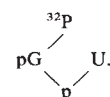
The most important result of this work is the detection of an RNA ligase in higher plants and the discovery of a novel mechanism for RNA ligation. In contrast to the known ligases<sup>2-5</sup>, the enzyme described here transfers the phosphate previously involved in a 3',5'-phosphodiester bond into a 2'-phosphomonoester before or during formation of a new 3',5'-phosphodiester bond. Such a structure revives old ideas about branched RNAs<sup>20</sup>. It is likely, however, that the 2'-phosphomonoester, 3',5'-linkage is either the end product or an



**Fig. 5** DEAE-cellulose TLC of digestion products of Ω<sub>c</sub>\*. Ω<sub>c</sub>\* was digested with: a, nuclease P<sub>1</sub>; b, nuclease P<sub>1</sub> followed by snake venom phosphodiesterase; c, nuclease P<sub>1</sub> followed by periodate oxidation and β-elimination<sup>18</sup>; d, RNase T<sub>2</sub>; e, RNase T<sub>2</sub> followed by nuclease P<sub>1</sub>; and f, RNase T<sub>2</sub> followed by nuclease P<sub>1</sub>, periodate oxidation and β-elimination. Chromatography was carried out at room temperature in 0.2 M ammonium formate, 9 M urea, 1 mM Na<sub>2</sub>EDTA, after a short pre-run in water<sup>32</sup>. An RNase A digest of poly(A, C) was used as a marker for net charges (see right-hand side of figure). In the case of nuclease P<sub>1</sub> digestion of Ω<sub>c</sub>\*, the net charge of the resulting oligonucleotide was confirmed by DEAE-Sephadex column chromatography<sup>33</sup> (data not shown). The corresponding products are:

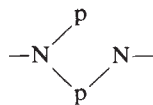


**Fig. 6** Sequence analysis of



Ω<sub>c</sub>\* was completely digested with nuclease P<sub>1</sub> and an aliquot of the labelled material was further treated with snake venom phosphodiesterase. The mixture of digested and undigested dinucleotide was separated by high voltage electrophoresis (first dimension) and homochromatography in 50 mM Homomix (second dimension)<sup>19</sup>. Xylene cyanol (XC) migrated 6 cm in the first dimension. For comparison, we have shown the sequence analysis of [5'-<sup>32</sup>P]pG-G-U (open circles). The dotted circle indicates the position of authentic pGp.

important intermediate of the ligation reaction. The 2'-phosphomonoester would render the newly formed linkage resistant to any reversible action of this ligase, such that any phosphatase, specific or nonspecific, could then generate a classical 3',5'-phosphodiester bond from the



structure. The exciting possibility exists that this type of ligation mechanism leading to, or through, a 2'-monophosphate, 3',5'-phosphodiester linkage, may be involved in eukaryotic RNA splicing. For example, it might operate during formation of yeast mitochondrial circular RNA<sup>21,22</sup> or the 0.4-kilobase ribosomal intron circle of *T. thermophila*<sup>23</sup>. Moreover, this type of ligation could occur during circularization of viroid RNA<sup>24,25</sup> from linear precursors. Interestingly, the splice sites in higher plants<sup>26</sup> are similar (although not identical) to those in other eukaryotes and eukaryotic viruses<sup>27</sup>.

The detailed ligation mechanism for this novel type of ligase is unclear, but it is probably a complex reaction involving several enzyme activities. For example, it is not known how the new 3'-

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## LETTERS TO NATURE

### Automated detection of variable objects on Schmidt plates

M. R. S. Hawkins

Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ, UK

Searches for variable objects on photographic plates have hitherto been largely confined to surveys for RR Lyrae stars; the work of Kinman *et al.*<sup>1</sup> and Plaut<sup>2</sup> is perhaps the best known. In these surveys large numbers of plates were 'blinked' in pairs to locate the variables, and typically had a limiting magnitude of about 19 and a limiting amplitude of 0.3 mag. The procedure is extremely time consuming, requires considerable expertise on the part of the measurer and necessitates a well matched sequence of plates. These surveys have greatly increased our understanding of the structure of the Galaxy, but further work has inevitably been limited by the effort required. In the field of quasars, Usher<sup>3</sup> systematically searched lists of preselected blue objects for variability, providing some important statistics on the frequency of variables, but without an automated search procedure it has not been possible to detect quasars systematically solely on the basis of their variability. I now report a procedure for automatically detecting variable objects and give a preliminary description of the type of object to be found. This technique should make it possible to extend the work on RR Lyraes and quasars down to a limit of at least  $B = 21$ , and to investigate the distribution of other classes of faint variable objects.

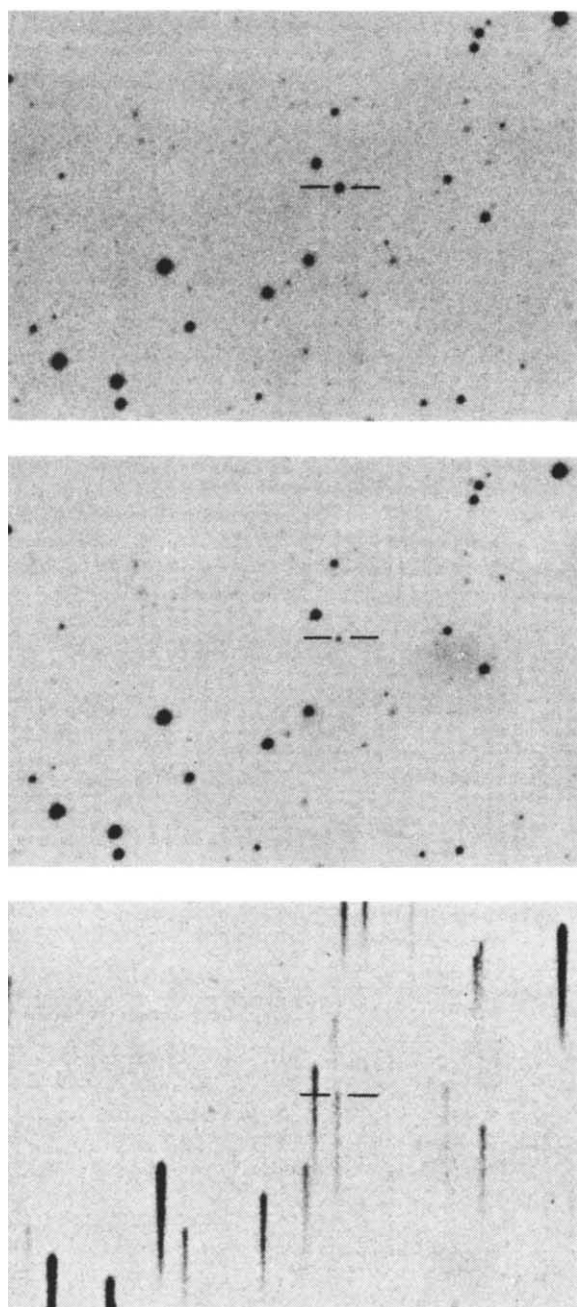
I have previously reported<sup>4</sup> the discovery of a distant supernova on a sequence of Schmidt plates (the observing programme and other observational details are also described in ref. 4). Seven of the plates, together with the early epoch J3376, have now been measured in the image analysis mode of the COSMOS measuring machine at the Royal Observatory, Edinburgh, which located and measured 120,000 images on each plate in an area of 16 deg<sup>2</sup>. The data sets were then combined to give a set of measures for each image. A 'master plate' was chosen and for all other 'secondary' plates a least-squares rotation, translation and scale transformation was evaluated. This transformation was applied to each master image, a small area on the secondary plate searched around the transformed position and the nearest image chosen as a provisional pair. This rather simple procedure has the undesirable result that the pairing will depend on which of the plates is chosen as master, and allows images to be double paired. To avoid this ambiguity a further algorithm was used which involved successively removing the closest matches, up to some limiting separation beyond which images were considered unpaired. In addition, an image which was not detected on any of the plates was discarded. This rather tight restriction resulted in the loss of an average of 1,000 images per plate, causing the sample size to drop from 120,000 to 110,000. Future work should examine images detected on only a few plates, as they undoubtedly contain some highly variable objects, but as most seem to be badly measured images of various types they are disregarded here.

The COSMOS threshold mapping mode precomputes a threshold above which images are detected, and outputs 20 parameters including positional, photometric and structural

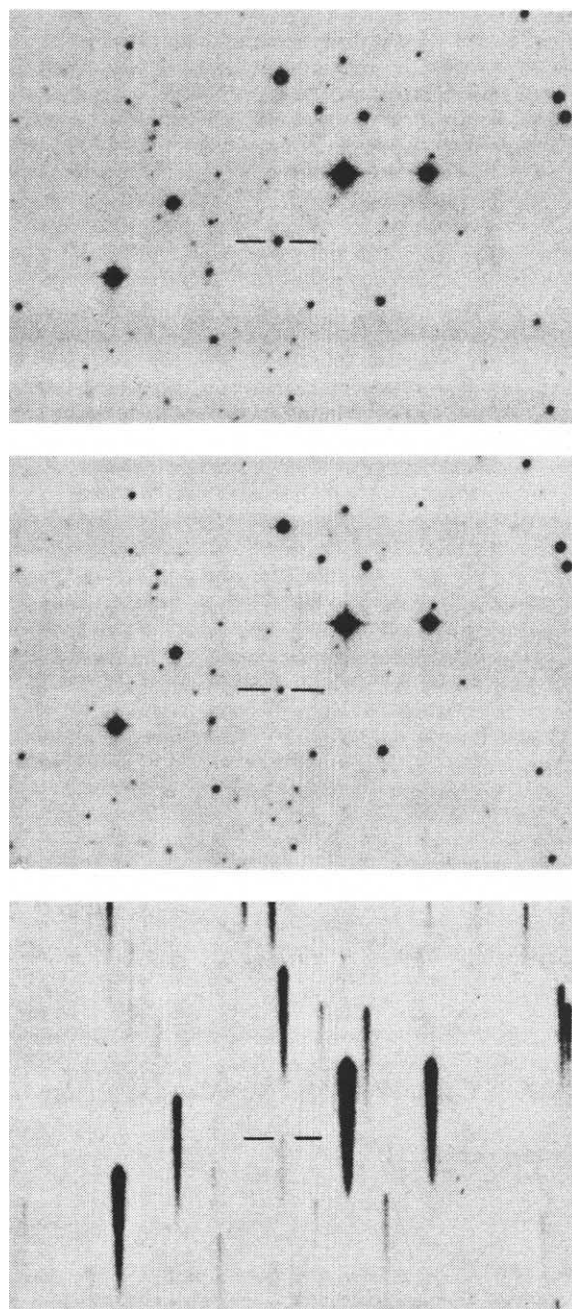


information for each image. Due to limitation in computer storage it was not practicable to carry through all parameters for each image as plates were successively paired. The procedure adopted was to record position and about four structural and photometric parameters for the master plate. For each secondary plate only one parameter was recorded, the sum of the intensities, as calibrated by step wedge, above the threshold. Due to thresholding and other effects this does not correspond to true magnitude, but is nonetheless a useful photometric parameter.

To detect variability it is clearly necessary to reduce all measurements to the same system, although this need not be true magnitude. Here, measurements on each plate were transformed to those on the master plate using a least-squares polynomial fit. Finally, all measurements were transformed to *B* magnitudes using an electronographic sequence. The effectiveness of this procedure was estimated by evaluating the r.m.s. variation in the measurements for each image, and plotting them



**Fig. 1** The variable object VI showing maximum and minimum luminosity and an objective prism spectrum.



**Fig. 2** The variable object V5 showing maximum and minimum luminosity and an objective prism spectrum.

as a histogram which peaked at 0.14 mag. This rather unsatisfactory value was greatly improved by making the transformation a function of position on the plate, and gave a r.m.s. peak at 0.08 mag, close to the practical limit for photometric accuracy on Schmidt plates. This error was essentially independent of magnitude to  $B = 21$ , the limit of the survey. This histogram of r.m.s. variation showed a pronounced tail to high values, including true variables and several categories of spurious variation which had first to be eliminated. These included close images resolved on only some of the plates, images located near the diffraction spikes or haloes of bright stars, and galaxies for which the calibration was not correct. Appropriate algorithms were devised to discard the various types of spurious variation, and a short list of variable candidates was produced. Examination of the plates by eye showed these to be mostly true variables.

Table 1 lists the variable objects detected, with details of their light curves and other information. The classification procedure

Table 1 Properties of variable objects

| Object no. | RA   |        |        | Dec          | $B_{\max}$ | $B_{\min}$ | r.m.s.* | Amplitude | Time scale | Class† |
|------------|------|--------|--------|--------------|------------|------------|---------|-----------|------------|--------|
| V1         | 21 h | 34 min | 45.2 s | -43° 55' 46" | 20.72      | 17.87      | 1.03    | 2.85      |            | BL     |
| V2         | 21   | 39     | 51.7   | -47 07 05    | 20.36      | 19.52      | 0.29    | 0.84      |            | BC     |
| V3         | 21   | 34     | 00.0   | -44 15 53    | 19.21      | 18.07      | 0.40    | 1.14      |            | QS     |
| V4         | 21   | 24     | 10.9   | -43 50 57    | 19.07      | 17.72      | 0.51    | 1.35      |            | BC     |
| V5         | 21   | 25     | 23.2   | -42 45 37    | 19.80      | 18.30      | 0.50    | 1.50      |            | BC     |
| V6         | 21   | 29     | 56.0   | -42 42 10    | 18.53      | 16.47      | 0.80    | 2.06      |            | QS     |
| V7         | 21   | 31     | 20.9   | -42 58 18    | 20.81      | 19.92      | 0.33    | 0.89      | LP†        | —      |
| V8         | 21   | 40     | 12.3   | -45 10 45    | 20.82      | 20.09      | 0.26    | 0.73      | LP         | —      |
| V9         | 21   | 31     | 03.7   | -44 51 44    | 17.09      | 16.15      | 0.42    | 0.94      |            | RR     |
| V10        | 21   | 20     | 00.9   | -43 47 51    | 19.11      | 18.09      | 0.35    | 1.02      |            | RR     |
| V11        | 21   | 20     | 45.3   | -43 23 51    | 19.35      | 18.25      | 0.37    | 1.10      |            | RR     |
| V12        | 21   | 30     | 04.2   | -43 07 45    | 20.37      | 19.50      | 0.30    | 0.87      | LP         | QS     |
| V13        | 21   | 38     | 54.6   | -43 41 31    | 20.69      | 20.10      | 0.21    | 0.59      | LP         | —      |
| V14        | 21   | 19     | 38.4   | -43 28 22    | 17.39      | 16.54      | 0.33    | 0.85      |            | RR     |
| V15        | 21   | 37     | 26.2   | -47 10 16    | 15.48      | 14.50      | 0.37    | 0.98      |            | RR     |
| V16        | 21   | 33     | 46.4   | -43 21 36    | 20.49      | 19.66      | 0.25    | 0.83      | LP         | QS     |
| V17        | 21   | 23     | 54.6   | -44 57 00    | 21.07      | 20.55      | 0.19    | 0.52      |            | —      |
| V18        | 21   | 30     | 16.1   | -45 23 14    | 21.06      | 20.47      | 0.20    | 0.59      |            | —      |
| V19        | 21   | 41     | 31.6   | -46 25 16    | 21.09      | 20.32      | 0.23    | 0.77      | LP         | —      |
| V20        | 21   | 23     | 45.5   | -43 08 48    | 21.02      | 20.49      | 0.17    | 0.53      | LP         | —      |
| V21        | 21   | 18     | 39.4   | -46 34 47    | 20.01      | 19.14      | 0.33    | 0.87      |            | RR     |
| V22        | 21   | 23     | 37.9   | -43 30 45    | 20.79      | 20.26      | 0.18    | 0.53      | LP         | QS     |
| V23        | 21   | 31     | 35.5   | -46 56 25    | 20.77      | 20.24      | 0.18    | 0.53      | LP         | QS     |
| V24        | 21   | 36     | 51.0   | -46 04 20    | 20.58      | 20.05      | 0.21    | 0.53      | LP         | BL     |
| V25        | 21   | 29     | 38.2   | -44 13 48    | 20.55      | 19.98      | 0.19    | 0.57      | LP         | BL     |
| V26        | 21   | 36     | 26.6   | -44 46 58    | 20.75      | 20.21      | 0.18    | 0.54      |            | —      |
| V27        | 21   | 37     | 25.9   | -45 29 29    | 20.44      | 19.86      | 0.19    | 0.58      | LP         | QS     |

\* The r.m.s. variation of the measures from the eight plates.

† LP indicates long-period variation implied by a systematic magnitude difference between the two early and six late epoch plates.

‡ Preliminary classification from a 1.2-m objective prism Schmidt plate copy taken out 2 months after the main run of plates and from the form of the variability. RR, QS and BL indicate RR Lyrae star, quasar and BL Lac candidates, respectively, BC indicates blue continuum objects and a dash indicates that the spectrum is too faint to classify at all.

used was as follows. Objects with an A-type spectrum on the objective prism plate and an amplitude  $>0.8$  mag were classified as RR Lyraes. Objects which either showed clear emission lines or a long, broken spectrum were classified as quasars. Objects with a long and apparently featureless spectrum have several possible classifications on that basis alone, although the associated variability suggests they may be BL Lac objects and this is the provisional classification given. Of the spectra which were bright enough to be seen on the objective prism plate this scheme accounted for all but three objects, apparently similar and characterized by a flat continuum with a sharp cutoff in the blue. The identity of these objects is not clear.

Figures 1 and 2 show Schmidt photographs of two of the objects detected, and indicate the full amplitude, together with the spectrum from the objective prism plate. Figure 3 shows the light curves of eight representative objects, isolated dots representing early and late epoch plates, and the joined points the main sequence of observations. The error bars show the mode of the r.m.s. variation described above, which gives a good indication of photometric error. The sample is considered to be essentially complete to an r.m.s. variation of 0.25 mag, as these highly variable objects are relatively easy to distinguish from spurious variables. For this complete part of the sample, the objective prism plate classifications from Table 1 give six RR

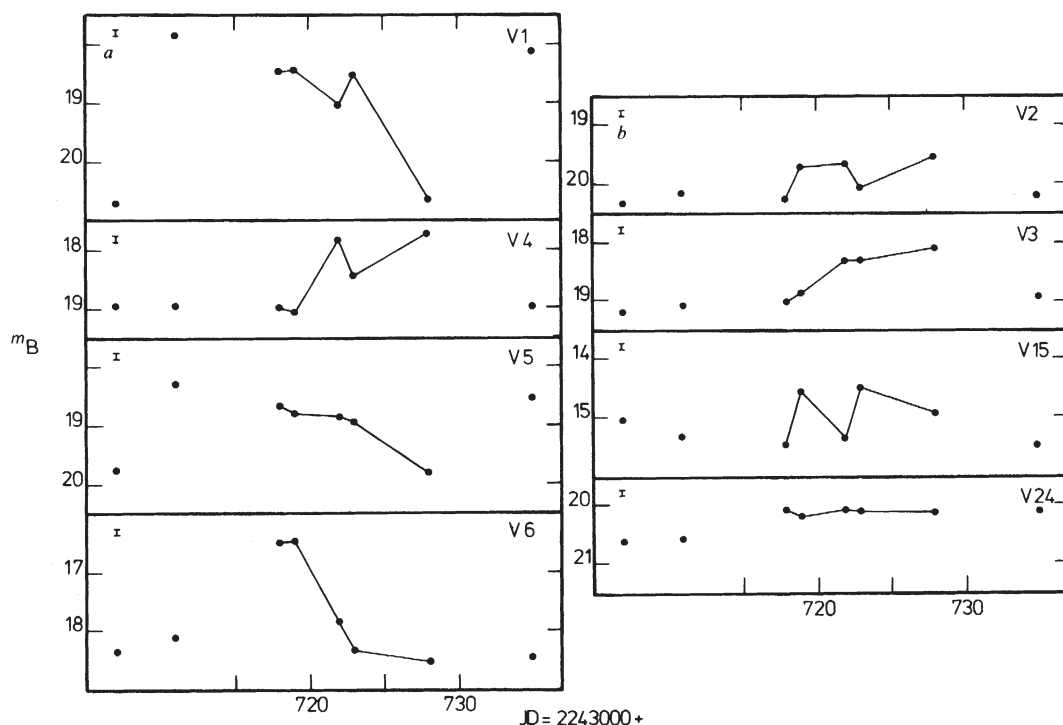


Fig. 3 a, Light curves of the most variable objects in the sample. b, Light curves of four representative objects from the sample. JD = 2243000.



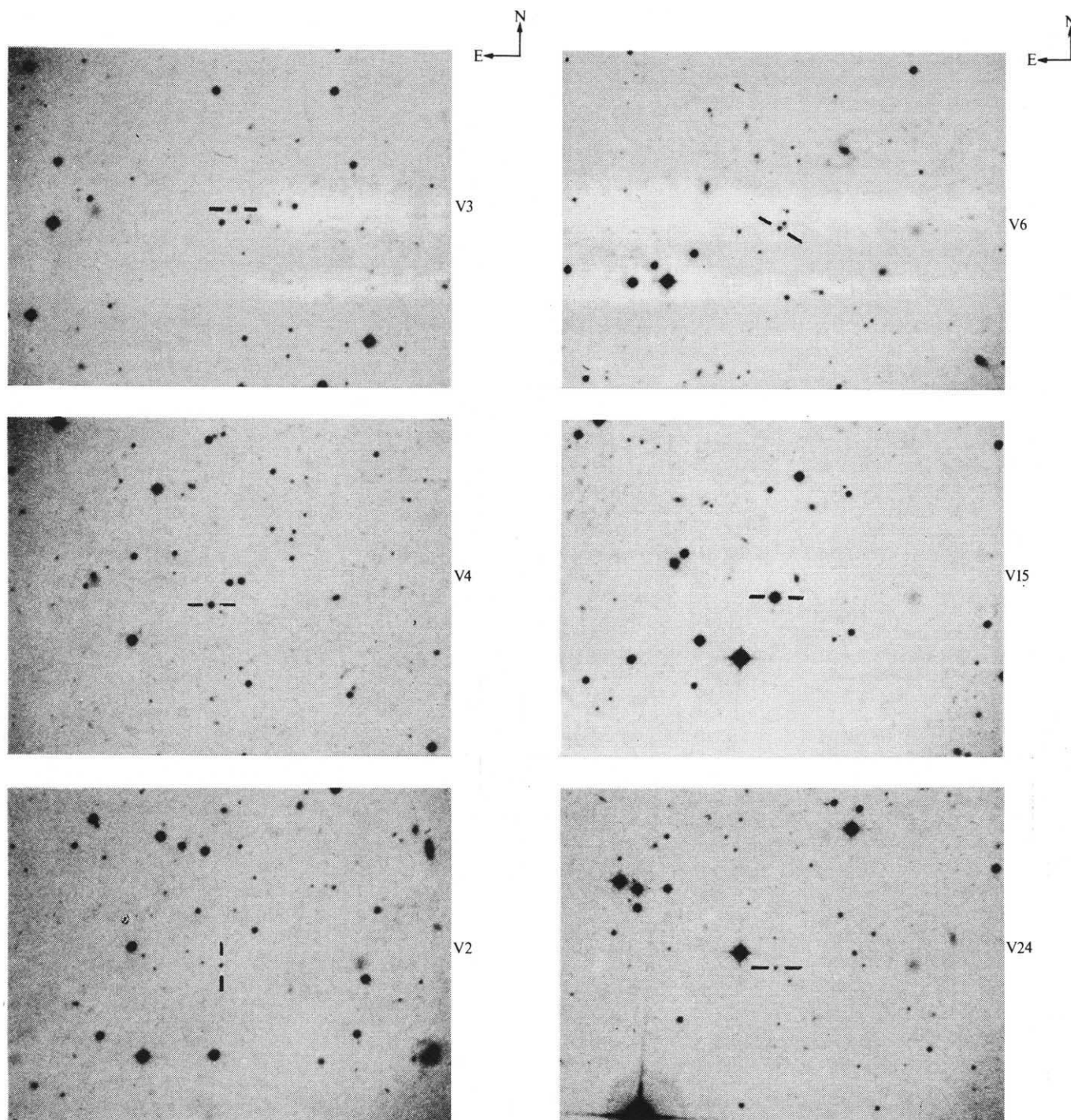


Fig. 4 Finding charts for the other objects with light curves.

Lyrae stars, three quasars, one BL Lac object, four objects with flat continua and a sharp cutoff, and two objects too faint to classify. Although the sample should be complete down to  $B = 21$ , of the five non-RR Lyrae objects with amplitude  $> 1$  mag only V1 has  $B_{\max} > 20$ . Surprisingly, although the limiting magnitude may well be excluding objects of great variability, there seems to be a real deficit of faint objects of more moderate amplitude. This cannot easily be explained by selection effects, which would tend to work the other way, giving apparently greater scatter to faint objects. In addition, there is a tendency for the fainter objects to be long-period variables in the sense defined in Table 1 legend.

The six RR Lyrae candidates form the most homogeneous class of objects detected. A cyclical pattern may be discerned in

the main sequence of observations for most of these objects (see Fig. 3b, third diagram). The faintest has a distance modulus of about 18.5, or 40 kpc, a surprisingly large value, and the ease with which these objects may be found should allow interesting studies of the galactic halo. Of the objects classified as quasars, the two brightest would fall into the category of OVV (optically violently variable), perhaps 1% of the quasars detectable on the objective prism plate. It is surprising that a larger number of faint violently variable quasars were not detected, as there seems to be no obvious significant observational selection effect against this. Confirmation of their classification and the analysis of a complete sample hopefully will clarify this point.

There is some uncertainty about all the BL Lac classifications, as they were on the basis of variability and an apparently



featureless blue spectrum. VI is particularly interesting, being extremely variable but not a Parkes radio source, and seems to be a strong candidate for a radio-quiet BL Lac object, of which no examples have so far been found. Polarization measures and a slit spectrum should allow a definite classification.

The last and perhaps most interesting class of object is characterized by a flat spectrum with a sharp cutoff. Tracing from the objective prism plate for different objects suggests that this cutoff does not occur at the same wavelength. The spectrum of V5 in Fig. 2 is a good example of the type, the break occurring at  $\sim 3,800 \text{ \AA}$ , which does not correspond to any zero-redshifted stellar feature. It is tempting to classify these objects as variable stars, but they do not seem to fit any known category. They appear blue on a B and R plate pair taken 1 h apart, which might suggest that they are dwarf novae, although the light curves are not consistent with this. The classification of these very variable objects must await a slit spectrum and perhaps other observations. Further analysis of the samples will be presented elsewhere.

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## Antiprotons in the cosmic radiation

P. Kiraly\*, J. Szabelski†, J. Wdowczyk†  
& A. W. Wolfendale‡

\* Central Research Institute for Physics, Budapest, Hungary

† Institute of Nuclear Research, Lodz, Poland

‡ Department of Physics, University of Durham,  
Durham DH1 3LE, UK

Golden *et al.*<sup>1</sup> have demonstrated that the measured ratio of the flux of antiprotons to protons ( $\bar{p}/p$  ratio) is significantly greater than expected from the commonly adopted 'leaky box' model of cosmic ray propagation, a model in which the  $\bar{p}$ 's are secondaries generated by the interaction of cosmic rays with the interstellar medium (ISM) in the Galaxy. At the energy at which measurements were made (kinetic energy  $\approx 8 \text{ GeV}$ ), disturbing effects due to interplanetary modulation are negligible and there is now general agreement<sup>2-5</sup> that the excess is a factor of 3 at least (an earlier calculation<sup>6</sup> of the expected  $\bar{p}/p$  which gave a result near to observation has been shown to be incorrect<sup>2-5</sup>). Bogolomov<sup>7</sup> found a similar but statistically less compelling enhancement at somewhat lower energies. A prominent characteristic of the  $\bar{p}/p$  ratio for galactic secondaries is a rapid fall with decreasing energy, below  $\approx 5 \text{ GeV}$ , due to kinematic factors. It was therefore remarkable that Buffington *et al.*<sup>8</sup> recently reported a ratio only a factor of 2.5 smaller at a very low kinetic energy (average  $\approx 0.2 \text{ GeV}$ ). This result, if correct, necessitates serious consideration of more exotic explanations. An important feature at these very low energies is the modulation of the flux by the interplanetary field and we have now examined this facet. We describe an improved calculation of the energy spectrum of  $\bar{p}$  expected for the standard leaky box model and show that even the enhanced secondary  $\bar{p}$  production in the 'closed Galaxy' model of Peters and Westergaard<sup>9</sup> fails by a wide margin to reproduce the high observed flux at low energies. A more radical departure from conventional thinking is to regard the observed  $\bar{p}$ s as primaries; a universal baryon-symmetric model and a black hole evaporation model are considered.

To compare the observed and calculated results, allowance must be made for solar modulation. These effects can be neglected above a few GeV, but are very important  $< 1 \text{ GeV}$ , particularly near the period of solar maximum (which applies to Buffington's point). Modulation in interplanetary space tends to

reduce the energy of cosmic rays entering the Solar System, the average adiabatic energy loss before reaching the region of the Earth being a few hundred MeV, depending on solar activity<sup>10,11</sup>. The effect is statistical, and the locally observed particles with a kinetic energy of  $\sim 200 \text{ MeV}$  must have had a wide distribution of interstellar energies. This distribution has been calculated by Urch and Gleeson<sup>11</sup> for solar maximum and minimum conditions for 'normal' interstellar spectra falling with increasing energy. If one neglects the partial exclusion of particles from the inner Solar System by energy-conserving convection-diffusion effects (previously thought to be the only cause of modulation), a lower limit is obtained for the demodulated flux, that is, for the interstellar flux generically related to the locally observed one.

Note that for the adiabatic energy loss process Liouville's theorem is valid (at least in an average sense), and thus the density in phase space is the same before and after deceleration. In contrast, the differential flux is proportional to the product of  $p^2$  and the phase space density, where  $p$  is the particle momentum. Using this relationship, the demodulated flux corresponding to Buffington's measurement was calculated for three possible values of the mean energy loss (400, 600 and 900 MeV). (Buffington *et al.*<sup>8</sup> used 600 MeV.) The highest value should apply only for peaked interstellar spectral shapes similar to those given in Fig. 1a,b. The dashed lines represent the effects of plausible deviations from Liouville's theorem.

Modulation theory is developing rapidly, and we refer here only to the recently discovered role of charge-dependent drift effects<sup>12,13</sup>. Positive and negative particles arrive through different regions of the heliosphere, those regions changing role at polarity reversals. Since 1971, it has been more difficult for negative particles to penetrate to the inner Solar System than for positive ones<sup>13</sup>, thus resulting in a lower electron/proton (and presumably also  $\bar{p}/p$ ) ratio than was the case in the previous solar cycle. The demodulated  $\bar{p}$  fluxes in Fig. 1 might therefore be even higher.

Interestingly, the ratio of observation to expectation for the  $\bar{p}$  flux (for model a or b) is essentially independent of the correction factor applied for modulation. There is, however, a slight dependence of the shape of the interstellar  $\bar{p}$  spectrum on the magnitude of the correction.

Figure 1 shows the results of model calculations for secondary  $\bar{p}$  production. The conventional explanation is in terms of production in the ISM; protons,  $\alpha$  particles and more massive cosmic rays interact with protons and heavier nuclei in the ISM to produce a variety of secondary particles including  $p$ ,  $\bar{p}$  and  $n$ ,  $\bar{n}$  pairs. In view of the importance of defining the conventional prediction, and because of previous discrepancies in predictions, we have made another independent estimate, which is indicated in Fig. 1a. The new calculations differ slightly from those given earlier<sup>3</sup> in that a new parameterization of the cross-sections is adopted.

$$E_p \frac{d^3\sigma}{dp^3} = N_p \frac{(1-x_R)^A}{(p_t^2 + 1.04)^{4.5}}$$

where  $x_R = E_p^*/E_p^*(\text{max})$  and the adopted parameters are  $A = 3 + 2.1 \log_{10} E_p$ ,  $N_p = 1.26$  for  $E_p < 4.8 \text{ GeV}$  and  $1.26 + 1.4 \log_{10} (E_p/48)$  for  $E_p > 4.8 \text{ GeV}$ .

The (important) spectrum of protons is as used previously<sup>3</sup>

$$j(T) = 2.0 \times 10^4 T^{-2.75} (4.26 T^{-0.876} + 1)^{-1} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ GeV}^{-1}$$

where  $T$  is the kinetic energy in GeV.

We consider that the invariant cross-sections are unlikely to be in error by  $> 15\%$  in the important energy region but the cosmic-ray spectrum is still uncertain by perhaps 25% at low energies. Table 1 compares the most recent predictions of the  $\bar{p}/p$  ratio at the important kinetic energies of 0.1 and 1.0 GeV derived recently<sup>4,5</sup>. Although there are differences between the derivations, which will be considered elsewhere, these are clearly small compared with the discrepancies with observation.

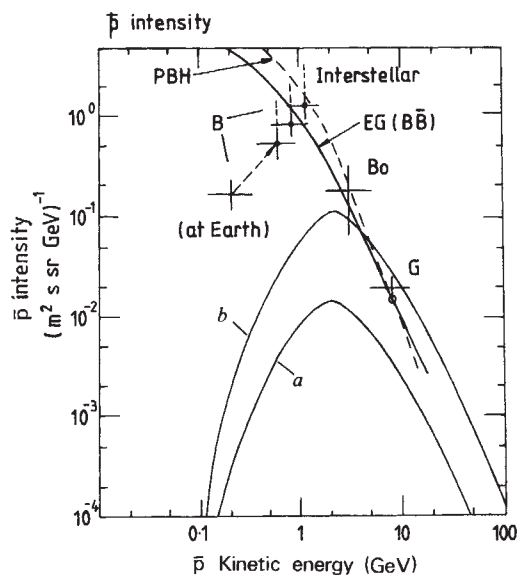
Curve a in Fig. 1 shows that the standard leaky box model is not applicable to the experimental situation at all; there is



insufficient latitude in the calculations to allow agreement. Curve *b*, which relates to the closed Galaxy model, gives agreement at 8 and 3 GeV but still shows a discrepancy at Buffington's point of a factor of at least 10. Although this conclusion may be modified by future developments, we consider a major change unlikely. It should be stressed again that the ratio of the demodulated observed value to that calculated is practically independent of the value accepted for the adiabatic energy loss. Also, the recently observed low  $\bar{e}/p$  ratio<sup>13</sup> (that is, depression of the intensity of negatively charged particles) suggests that the discrepancy may be larger. Constraints are also introduced by the fact that the local  $e^-$  spectrum in the 100-MeV region is already below expectation<sup>14</sup>. The strongest objection to the closed Galaxy model is based on its prediction of an excess of secondary positrons<sup>15</sup> of 4–9, the value depending on the details of the model, at 3 GeV.

The  $\bar{p}$  observations clearly favour a power-law type spectrum similar in shape to the  $p$  spectrum, but scaled down by a factor of  $\sim 2 \times 10^3$ . Such spectra would naturally arise either as a result of acceleration from a nearly thermal  $\bar{p}$  component, or from  $\bar{p}$  emission by evaporating primordial black holes.

It is apparent that the first possibility could not easily be realized in our Galaxy, because of the huge annihilation radiation levels involved. In a baryon-symmetric universe ( $\bar{B}\bar{B}$  model), however, matter and antimatter might be separated on the supercluster scale, and annihilation radiation would still be on an acceptable level, while cosmic rays leaking out of galaxies might find their way into galaxies of the opposite baryon charge. Such a scenario, considered by us previously<sup>3</sup>, has recently been described by Stecker<sup>16</sup> and claimed to be compatible with the  $\bar{p}$  observations.



**Fig. 1** Secondary antiproton flux predictions. *a*, Leaky box model with  $\lambda = 5 \text{ g cm}^{-2}$ ; *b*, closed Galaxy model. The displacement of the Buffington *et al.*<sup>8</sup> point (marked B) is to allow for solar modulation. The points correspond to mean adiabatic energy losses of 400, 600 and 900 MeV. The horizontal error bars represent the energy intervals from which  $\sim 70\%$  of the measured flux at Earth is expected. The highest value (900 MeV) would be unreasonably high for protons or for primary  $\bar{p}$ s with a power-law spectral shape, but is quite possible for the peaked spectra of secondary  $\bar{p}$ s. The dashed vertical bars represent plausible deviations from Liouville's theorem. Bo denotes Bogomolov *et al.*<sup>7</sup>; G denotes Golden *et al.*<sup>1</sup>; EG( $\bar{B}\bar{B}$ ) denotes the prediction for extragalactic  $\bar{p}$ s generated in antigalaxies in a baryon-antibaryon symmetric universe normalized at 9 GeV. If there is a significant galactic wind the  $\bar{p}$  intensities will fall off with falling energy ( $< 1 \text{ GeV}$ ). PBH denotes the prediction for the exploding primaeval black hole model (quark version) normalized as above. The remarks about the influence of a galactic wind made in the last paragraph apply here too if, as is likely, many of the BHs are in the galactic halo.

**Table 1** Comparison of  $\bar{p}$  production rates for  $\lambda = 5 \text{ g cm}^{-2}$  traversal by cosmic rays in the Galaxy

| $E_{\bar{p}}$<br>(GeV) | Stephens <sup>4</sup> /ours | Data<br>Tan & Ng <sup>5</sup> /ours |
|------------------------|-----------------------------|-------------------------------------|
| 0.2                    | 1.15                        | 1.32                                |
| 1.0                    | 1.55*                       | 1.27                                |

This table indicates the extent to which different workers make differing predictions; the uncertainty in production rate is seen to be very much less than the discrepancy between observation and conventional expectation (curve *a* in Fig. 1).

\* This value falls to 0.94 when we take out what we believe is an error in Stephens' analysis.

In the absence of a clear indication of what extragalactic  $\bar{p}$  flux might be expected on the  $\bar{B}\bar{B}$  model, we have normalized the prediction to observation. The normalization corresponds to the assumption that our Galaxy is underefficient by a factor of  $\sim 20$  in producing cosmic rays, assuming equal densities of galaxies and antigalaxies with  $\rho = 10^{-2} \text{ Mpc}^{-3}$  (the 'usual' density of galaxies being  $\sim 2 \times 10^{-2} \text{ Mpc}^{-3}$ ). Such a factor is perhaps not too unexpected in view of the fact that the extragalactic  $\gamma$ -ray flux above 100 MeV is about 25 times that expected from 'normal' galaxies<sup>17</sup> in the absence of antigalaxies.

However, an explanation in terms of the  $\bar{B}\bar{B}$  model has some drawbacks, aside from problems associated with separating matter and antimatter at early epochs. (1) The measured upper limit to the  $\bar{\text{He}}/\text{He}$  ratio ( $2.2 \times 10^{-5}$  at the 95% confidence level)<sup>8</sup> is smaller than expected if  $\bar{\text{He}}/\bar{p}$  in  $\bar{B}$  galaxies is the same as  $\text{He}/p$  in our Galaxy. Although one might suggest fragmentation of  $\bar{\text{He}}$  near the sources<sup>14,16</sup>, by interactions with a high density of matter or radiation, the associated  $\gamma$  flux (from  $e^+e^-$  production in nucleus-photon interactions) seems too high. Certainly, if interactions with gas are responsible, the 'grammage' of extragalactic particles ( $\bar{p}$ ,  $\bar{\alpha}$ ...) would need to be  $\sim 100 \text{ g cm}^{-2}$  to attenuate  $\bar{\alpha}$  sufficiently and the isotropic  $\gamma$ -ray flux would be too high by a factor of  $\sim 100/5 = 20$  (the grammage in our own Galaxy is  $\sim 5 \text{ g cm}^{-2}$  and, as we have pointed out, the isotropic  $\gamma$ -ray flux is already accounted for by interactions in the external galaxies). (2) Virtually straight line propagation is necessary in extragalactic space; a chaotic field even as low as  $10^{-12} \text{ G}$  would cause diffusive motion and prevent  $\bar{p}$  from beyond the Virgo cluster arriving at the Earth. (3) The (likely) galactic wind would preclude entry into the Galaxy.

The question of the magnetic field in extragalactic space remains open but it is unreasonable to expect a mean field  $\ll 10^{-12} \text{ G}$ . If the energy densities of magnetic fields and kinetic energy of gas clouds approach equipartition, we might expect a mean field in clusters of  $\sim 10^{-8} \text{ G}$  (ref. 18); similarly, with a mean gas density in the Universe overall of  $\sim 10^{-8} \text{ atoms cm}^{-3}$ , the corresponding field is  $\sim 10^{-10} \text{ G}$ . In both cases, the velocities of galactic and extragalactic gas clouds are assumed to be similar. The source of the field would be either primordial or (and this is very likely) supernovae ejecta which escape from galaxies. We believe that there is probably a sufficiently large field within clusters to confine low-energy (GeV) particles to their parent clusters—and in turn to keep out particles from elsewhere. Thus, it is very difficult to accept the  $\bar{B}\bar{B}$  model despite its apparent success in giving the correct spectral shape.

We consider two other models—both galactic—to be contenders. The first is the galactic centre explosion model of Khazan and Ptuskin<sup>19</sup> which we have already considered in an attempt to explain the  $\bar{p}$  results at 8 GeV and other features<sup>20</sup>. One might postulate an *ad hoc* model in which  $\bar{p}$ s are generated as secondaries in an explosion then slowed down by adiabatic expansion, and in which a low  $\text{He}/\text{He}$  ratio is also generated, but this seems contrived, and—because of the secondary nature of  $\bar{p}$ s—would probably give too few  $\bar{p}$ s at low energies. It seems easier to postulate an excess of  $\bar{p}$ s in the explosion itself. For example, an exploding massive object might produce  $p\bar{p}$  pairs, the  $p$  being augmented by more conventional sources in the Galaxy to give the observed  $\bar{p}/p$  ratio. Although it cannot be

excluded, we are unaware of any quantitative predictions for a model of this type.

The second galactic model involves evaporating primordial black holes (PBHs), first suggested by Hawking<sup>21</sup> and later elaborated by other workers (see, for example, refs 22–26). PBHs with original masses below  $\sim 5 \times 10^{14}$  g have already evaporated (if created in the early Universe), but this low mass and high temperature part is repopulated in a quasi-equilibrium fashion as a result of mass loss of PBHs with original masses slightly above  $5 \times 10^{14}$  g. This quasi-equilibrium range might give a sufficient contribution to the  $\bar{p}$  flux in the Galaxy without infringing other observational limits. Note that the initial number spectrum of PBHs should fall sharply with increasing mass (following a power law with negative exponent of 2 to 3, depending on the equation of state of the early Universe)<sup>27</sup>. Thus, the contribution of larger masses to  $e^+$  and  $\gamma$  production should be negligible.

The basic idea is that a PBH radiates like a black body of temperature  $\theta = \hbar c^3 / (8\pi Gmk) = 10^{26} m^{-1}$  K. Following Carr<sup>23</sup> and others, the time-integrated energy distribution of all the particles emitted by a black hole of initial mass  $m$  is  $N(E) \approx 10^{54} E^{-3} \text{ eV}^{-1}$  (for  $E > 10^{22} m^{-1} \text{ eV}$ ), and there is thus the possibility of explaining the observed  $\bar{p}$  spectrum, in the relativistic region, where the simple power law should be valid.

We will now use published work to derive the expected  $\bar{p}$  energy spectrum at the 'low' energies of particular relevance here ( $E \approx 1 \text{ GeV}$ ), the spectrum not having been given explicitly elsewhere, and derive the expected fluxes of electrons and  $\gamma$  rays from PBH. Clearly the last mentioned should be at least not inconsistent with observation if the model is to be taken seriously.

Carr<sup>23</sup> has given the fraction of energy going into different 'particles' (gravitons, photons, neutrinos, electrons and nucleon-anti-nucleon pairs) as a function of initial PBH mass. For example, in the important mass range  $10^{13.5} < m < 10^{14}$  g, the fractions are, respectively  $< 1\%$ ,  $11\%$ ,  $40\%$ ,  $25\%$  and  $12\%$  (the last value is now considered to be too high; S. W. Hawking, G. W. Gibbons and B. J. Carr, personal communication) but the following arguments are not seriously affected). Page<sup>26</sup> has given the average emission rate and power of particles as a function of their mass for different black hole masses and the two sets of data have been used to estimate the spectra for  $\bar{p}$ ,  $e^+$  and  $\gamma$ . The calculations have been made for both the bootstrap and elementary particle (Ep) models, the latter giving rise to a spectrum with higher mean energy because with this model the PBH continues to a lower mass before evaporating catastrophically. The calculation is approximate insofar as we assume as spectral form for the antiprotons the expression  $N(E)_p = A(E + E_0)^{-3}$ , where  $E$  is the kinetic energy and  $E_0$  and  $A$  are constants determined by the method:  $E_0 = 1.0 \text{ GeV}$  and  $A = 5.4 \times 10^{36} \text{ GeV}^2$  for the Ep model.

Figure 1 shows this prediction, normalized as before at  $9 \text{ GeV}$ . Again, as with the extragalactic  $\bar{B}\bar{B}$  model, the spectral shape is consistent with experiment.

Normalization of the  $\bar{p}$  flux leads to a specific prediction for the flux of electrons ( $e^+ + e^-$ ):  $N(E)_e = 8.4 E^{-3} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ GeV}^{-1}$ , neglecting energy losses. This spectrum is unlikely to be affected by energy losses until above several GeV and can thus be compared with experiment. Such a comparison (see, for example, ref. 28) shows that at  $1 \text{ GeV}$ , where the measured intensities are not badly affected by solar modulation, the PBH prediction is below observation by a factor of 11; at higher energies the factor will be even bigger and there is no inconsistency. It is interesting—and perhaps important—that in the region of  $100 \text{ MeV}$ , where direct measurements of the electron spectrum are not available, the PBH intensity is higher by a factor of 3 than the intensity expected from an extrapolation of the measured spectrum (the extrapolation is supported by data on the electron-positron ratio<sup>29</sup>). This factor helps considerably in explaining the well known result<sup>30</sup> that synchrotron and  $\gamma$ -ray data need electron intensities in this energy region higher by a factor of 3–10.

Another point in favour of the PBH model is that the observed  $e^+/(e^+ + e^-)$  ratio tends to 0.5 at energies  $< 100 \text{ MeV}$ , which is just where PBH electrons should predominate and will give this ratio.

The predicted flux is small compared with observation, both for  $\gamma$ -ray bursts and for the galactic (and extragalactic) continuum, so that  $\gamma$ -ray flux from PBH is no problem. If the black holes are assumed to be confined to galaxies and their halos, the antiproton data reduce the upper limit to their density by a factor of 30 compared with the data on the extragalactic  $\gamma$ -ray flux used previously to evaluate the limit.

No  $\bar{\text{He}}$  nuclei are expected from PBHs and thus the stringent observational limits of Buffington *et al.*<sup>8</sup> are satisfied.

Thus, none of the models considered is free of difficulties, but the exploding primaeval black hole model has attractive features; it would be useful to have more specific predictions of the expected spectra of antiprotons and electrons on this model. It is clearly essential to re-measure the  $\bar{p}$  flux below  $1 \text{ GeV}$  with better statistics and with more refined equipment. After the polarity reversal of the interplanetary magnetic field, the  $\bar{p}/p$  ratio might be enhanced, thus giving a check and leading to the possibility of making new definitive measurements in this exciting and potentially revolutionary area of cosmic ray research.

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## Solar activity and solar neutrino flux

L. J. Lanzerotti & R. S. Raghavan

Bell Laboratories, Murray Hill, New Jersey 07974, USA

Among discussions of the implications of the Davis experiment<sup>1</sup> to detect solar neutrinos, there have been several suggestions that manifestations of solar activity are related to the flux of the neutrinos<sup>2–4</sup>. For example, it has been implied that (1) the physical processes in the deep interior of the Sun are related to, or even produce, the solar activity (exemplified by solar flares and sunspots) observed in the photosphere<sup>2,4</sup>; or that (2) some of the observed neutrinos are produced in nuclear reaction processes caused by energetic particles in solar flares and solar active regions<sup>3</sup>. These suggestions have been speculative because the available solar neutrino data covered only a fraction of a solar cycle. We now re-examine the proposals using data over a significantly longer time base and find no evidence for either of these possibilities.



We have investigated the temporal relationships between the detected neutrino fluxes and solar activity over the decade 1970–79. We have taken as representative of the solar activity the monthly mean Zurich sunspot numbers<sup>5</sup> and a monthly solar flare activity index based on the total area of solar active areas<sup>5,6</sup>. All solar indices of this type are based on the visible hemisphere of the Sun. Monthly values take into account a complete solar rotation, although a large flare on the invisible disk that might contribute to hypothesis (2) could be missed. The active area index was specifically used because it was speculated that if this hypothesis held, then the total area of the energetic solar activity might provide a better measure of surface solar processes than the more indirect resultant, geomagnetic activity<sup>3</sup>. Sunspot number was chosen as representative of solar activity because of its availability and wide usage and because it has been suggested<sup>4</sup> that, for the period 1970–75, there was a relationship between sunspot number and neutrinos.

The decade of data analysed here encompasses approximately one complete solar cycle: beginning with the declining phase of the twentieth cycle it passes through solar minimum and comes to the growing phase of the present, twenty-first cycle. Figure 1b shows as a function of time from 1970 to 1979, the neutrino fluxes expressed in terms of <sup>37</sup>Ar atom production from the <sup>37</sup>Cl in the Davis detector. The solid horizontal lines indicate the most probable values as well as the time intervals over which each datum was acquired. The vertical lines indicate the uncertainty in the measurements. The numbers can be related to solar neutrino units (1 SNU = 10<sup>-36</sup> neutrino captures per target particle per s) by subtracting a muon background (0.08 atoms per day) and multiplying by 5.31. It is evident that there is a wide range of variability in the <sup>37</sup>Ar production during this 10-yr period about a mean of ~0.5 atoms per day. Particularly noticeable is a minimum in fluxes during 1976, increasing to higher values in 1977, and tending to decrease overall after that time.

The Zurich sunspot numbers and the solar active area values are shown in Fig. 1a, c. The solar active area and sunspot number indexes show a similar behaviour during this 10-year period.

The three time series show that there is no overall relationship between the solar activity and the neutrino flux. The neutrino flux does not follow in general the decrease of solar activity in cycle 20 nor does the flux follow the sharp increase in activity with the onset of cycle 21. Such a relationship, originally suggested by Sheldon<sup>2</sup>, was shown not to hold in the declining phase of cycle 20 (ref. 4). Indeed, for the beginning of cycle 21, except for two data points, the overall trend of the neutrino flux since mid-1977 has been a decrease, opposite to the sharp increase in solar activity.

Thus, from the data examined, the neutrino flux does not result from solar activity processes on the surface of the Sun. If the neutrinos result from nuclear processes deep in the interior of the Sun, the results would also suggest that the approximately 11-yr solar activity cycle is not closely related to a nuclear burning cycle.

Much interest has centred recently on using solar oscillations as a method of 'solar seismology' to study the interior of the Sun<sup>7</sup>. Oscillations of 5-min period are definitely established<sup>8,9</sup>; oscillations of ~160 min period, which would probe deeper into the Sun, are less well established<sup>10-13</sup>. Discussions abound of possible 'quasi-biennial' variations in solar activity<sup>14</sup> which may appear as effects on Earth as quasi-biennial variations in such parameters as geomagnetic activity<sup>15</sup> and temperature<sup>16</sup>. However, the quasi-biennial variation in temperature has been shown to be very nonstationary and dependent on location<sup>17</sup> for eastern US and British sites. Sakurai<sup>4</sup> showed that the sunspots in the declining phase of solar cycle 20 exhibited a quasi-biennial variation for nearly two cycles between 1970 and 1975; the neutrino flux seems to exhibit a similar periodicity during this interval, with perhaps a 4-month lag<sup>4</sup>.

The solar activity indexes between 1976 and 1979 do not show evidence for a continuation of a quasi-biennial variation.

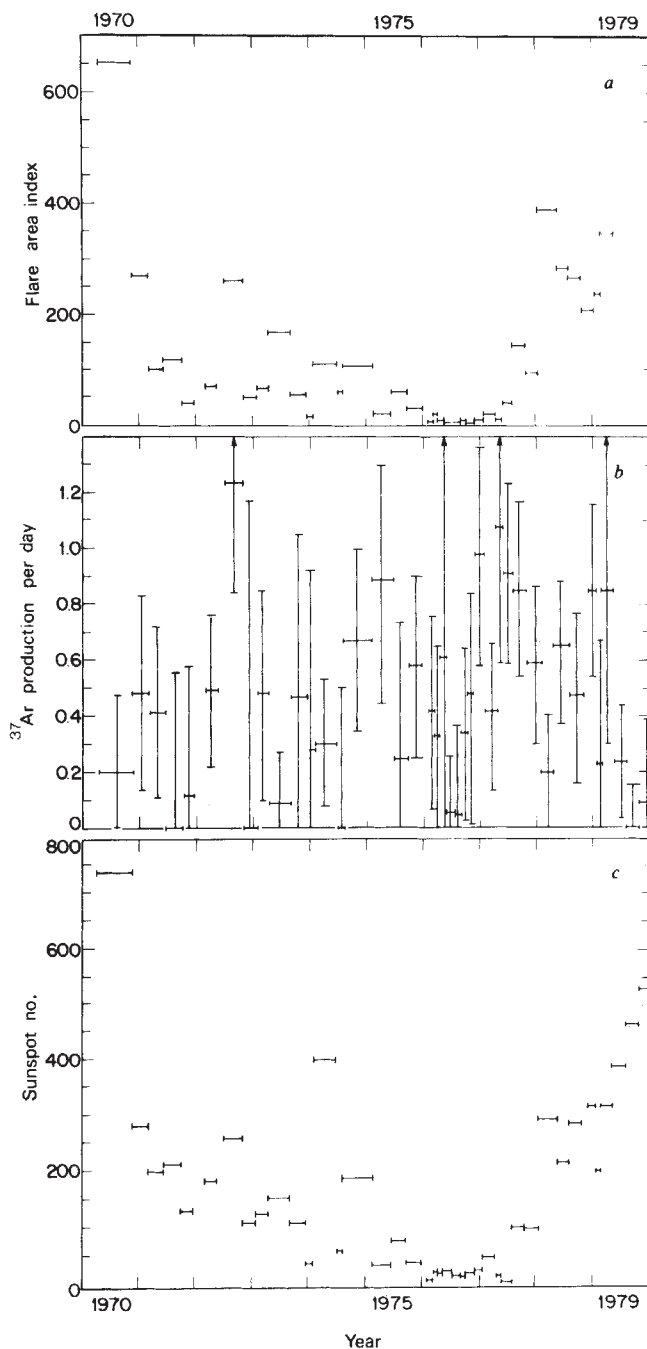


Fig. 1 <sup>37</sup>Ar production per day as a function of time for the interval 1970–79 (b). a, The solar flare area index; c, the sunspot number, for the same time intervals over which each <sup>37</sup>Ar point was acquired.

This would suggest that such an oscillation, if real, is not stationary; its possible relationship to interior solar processes is unclear.

The neutrino fluxes in the increasing phase of the twenty-first solar cycle do not exhibit any relationship to the solar indexes. The fluxes also do not exhibit any quasi-periodicity that might be attributable to interior solar processes during this interval.

We therefore find no significant relationship between solar activity and the neutrino flux on either a long-term (solar cycle) basis or for shorter terms (for example, 2 yr). This suggests that the internal nuclear mechanisms in the Sun are not clearly related to solar activity as expressed by the parameters discussed here. That is, the basic physical processes involved with producing photosphere manifestations of solar changes (such as solar magnetic fields; bright regions) have an immediate source

different than the internal nuclear sources. Our finding also suggests that the production of solar neutrinos by energetic particles involved in solar activity is not a significant contributor to the neutrino flux measurements now in progress.

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## Solar flare irradiation records in Antarctic meteorites

J. N. Goswami

Physical Research Laboratory, Ahmedabad 380009, India

**Petrographical studies<sup>1,2</sup> indicate that most meteorites collected from the Antarctic ice sheets are L and H chondrites of different metamorphic groups. Achondrites and iron meteorites constitute <5% of the total collection of about 1,600. Radio-nuclide<sup>3-9</sup>, specifically <sup>53</sup>Mn, studies have suggested a multiple exposure history for some of the meteorites involving shielded exposure to cosmic rays either in large asteroidal-size parent bodies, from which the meteorites were ejected, or in relatively smaller-size objects that broke up in space in recent times<sup>3</sup>. Relatively fewer Antarctic meteorites have been studied for their noble gas content<sup>10-13</sup> and records of cosmic ray heavy nuclei tracks (ref. 14 and N. Bhandari, unpublished results); there has been no evidence of solar flare tracks and solar wind noble gases in the analysed samples. I now report the first observation of solar flare heavy nuclei tracks in Antarctic meteorite samples. Two interior specimens of sample 77216, an L-3 chondrite, contain track-rich grains indicating their exposure to solar flare irradiation before compaction of this meteorite. Preliminary noble gas data also indicate the presence of solar-type gases. Results of nuclear track studies of other Antarctic meteorite samples are presented.**

Solar flare tracks and solar wind noble gases are expected to be seen in certain meteorite types, the gas-rich meteorites, due to the precompaction exposure of individual components of these meteorites to solar flare and solar wind irradiation. Identification of such meteorites is important because: (1) they allow study of solar flare activity during different epochs in the past<sup>15,16</sup>; and (2) analyses of their solar wind and solar flare irradiation records help to elucidate the regolith dynamic processes operating in the meteorite parent bodies and the evolution of small objects in the early Solar System<sup>15,17-23</sup>.

The fraction of gas-rich meteorites among the different meteorite types varies widely<sup>21</sup>. Of the non-Antarctic L- and H-group chondrites analysed for their noble gas contents, ~3% and 15% respectively have been found to be gas rich. A systematic study was therefore initiated to look for Antarctic

meteorites having precompaction solar flare irradiation records, using conventional nuclear track techniques<sup>24-26</sup>. Eight L and H chondrites of low metamorphic grades were initially analysed. The sample details and the results of the track studies are summarized in Table 1. Most of the results are based on analysis of olivine grains, the major mineral species in the selected samples. Pyroxene grains were also analysed in two cases.

Two interior specimens of sample 77216, an L-3 chondrite, contain a few per cent of olivine grains with track densities much higher than the average background track density measured in the rest of the analysed grains. None of the 16 pyroxene grains analysed had been irradiated. Examples of track-rich grains are shown in Fig. 1, and the frequency distribution of track density for a set of representative grains from the sub-samples of 77216 is shown in Fig. 2. The average background densities of  $\sim 2 \times 10^6 \text{ cm}^{-2}$  and  $\sim 4 \times 10^6 \text{ cm}^{-2}$  in the two specimens (77216, 18, and 77216, 24) probably relate to the difference in their shielding depths within the original meteorite. The track densities in the irradiated grains range from  $1.5 \times 10^7 \text{ cm}^{-2}$  to  $\geq 10^8 \text{ cm}^{-2}$ , and include grains both with and without a track gradient, indicating their precompaction solar flare irradiation in different shielding conditions. The percentage of track-rich olivine grains varies between the two specimens, probably reflecting an inherent spatial variation in the abundance of track-rich grains in the meteorite itself, as is the case with many other non-Antarctic gas-rich meteorites<sup>23</sup>.

Aliquots of the samples used for nuclear track studies were prepared for noble gas and radionuclide measurements. Preliminary data of mass-spectrometric analysis of sample 77216, 18 (C. M. Nautiyal and T. R. Venkatesan, personal communication) show a large excess of noble gases, with a <sup>20</sup>Ne/<sup>22</sup>Ne ratio of  $\geq 10$ , clearly indicating the presence of solar-type noble gas. Stepwise heating mass-spectrometric analysis of different size fractions is being carried out, and I also intend to investigate whether precompaction solar flare irradiation records are present in the Antarctic meteorite samples 77215, 77217 and 77252 which are suspected, on the basis of petrographical data as well as their positions in the Antarctic ice sheet at the time of recovery, to belong to the same fall as 77216.

Only five non-Antarctic L-group chondrites, which constitute about 2.5% of the L-group meteorites analysed for their noble gas contents, contain solar-type noble gases. The Antarctic meteorite sample 77216 thus constitutes a significant addition to the gas-rich meteorites of this group. Furthermore, detailed study of the Antarctic L and H chondrites should show whether the large excess of gas-rich H over gas-rich L chondrites found among non-Antarctic meteorites is also true of the Antarctic meteorites. An affirmative result will indicate significant

**Table 1** Nuclear track data for Antarctic meteorites

| Sample no. | Meteorite type | Average track density ( $\text{cm}^{-2}$ ) | Shielding depth (cm) | Cosmic-ray exposure age (Myr) |
|------------|----------------|--------------------------------------------|----------------------|-------------------------------|
| 77003, 36  | H-3            | $3.8 \times 10^6$                          | $4 \pm 0.2$          | 8*                            |
| 77015, 3   | L-3            | $\sim 10^4$                                | $22 \pm 1$           | 3.5†                          |
| 77216, 18  | L-3            | $10^6 - 10^8 \ddagger$                     | —                    | —                             |
| 77216, 24  | L-3            | $10^6 - 10^8 \ddagger$                     | —                    | —                             |
| 77230, 21  | L-4            | $2.5 \times 10^5$                          | —                    | —                             |
| 77260, 13  | L-3            | $1.4 \times 10^5$                          | $9 \pm 0.4$          | 1.9†                          |
| 77262, 12  | L-4            | $5 \times 10^5$                            | —                    | —                             |
| 77304, 18  | LL-3           | $2 \times 10^4$                            | —                    | —                             |

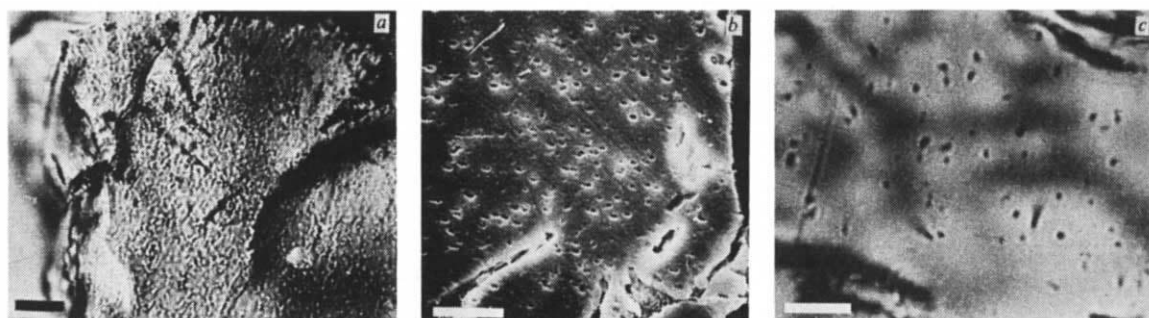
Shielding depth was calculated following the procedure of Bhat-tacharya *et al.*<sup>27</sup>. The errors in track density measurements only are considered in these estimates.

\* Based on <sup>53</sup>Mn data<sup>4</sup>. Production rate of <sup>53</sup>Mn used =  $414 \text{ d.p.m. kg}^{-1} (\text{Fe} + 1/3 \text{ Ni})$ .

† Based on preliminary <sup>21</sup>Ne data (N. Takaoka, personal communication). Production rate of <sup>21</sup>Ne used =  $0.31 \times 10^{-8} \text{ cm}^3 \text{ STP g}^{-1} (^{22}\text{Ne}/^{21}\text{Ne} = 1.11)^6$ .

‡ These two samples contain track-rich grains.





**Fig. 1** Optical and scanning electron photomicrographs of track-rich olivine grains, with and without a track gradient, in Antarctic meteorite 77216 (a, b). The tracks are produced by the iron group of nuclei in solar cosmic rays. A few long tracks due to the trans-iron group of nuclei can be seen in the optical photomicrograph (a). A grain having normal background track density of a few times  $10^6 \text{ cm}^{-2}$  is shown in c. Scale bar, 10  $\mu\text{m}$ .

differences in the evolution and irradiation history of the parent material of these two types of chondrite.

The measured track densities in the other Antarctic meteorites analysed in the present work range from  $10^4 \text{ cm}^{-2}$  to  $4 \times 10^6 \text{ cm}^{-2}$ . These values are within the range observed in non-Antarctic L-group meteorites. Evaluation of the track data in terms of the shielding depth of the analysed samples in the original (pre-atmospheric) meteorites and their masses requires a knowledge of the cosmic-ray exposure ages of these meteorites. Exposure age data, based on  $^{53}\text{Mn}$  and  $^{21}\text{Ne}$  measurements, are available only for samples 77003, 77015 and 77260. The shielding depths of these three samples, based on the track and exposure age data, are given in Table 1. The errors in the shielding depth estimate are  $\leq 10\%$  and result from the combined effect of errors in track density measurements, and errors in the deduced exposure ages due to the use of approximate shielding correction factors in obtaining production rates of  $^{53}\text{Mn}$  and  $^{21}\text{Ne}$ . In the case of 77003, where the sub-sample 77003, 36 analysed for nuclear track records represents a near-crust sample, a lower limit to the pre-atmospheric mass of 8.0 kg is obtained. This estimation is based on an exposure age of 8 Myr for the meteorite, and the following assumptions: (1) the single recovered fragment weighing 0.78 kg represents the post-atmospheric mass of this meteorite, and (2) the ablation is symmetric. Weakly bound lower limits of 220 kg and 30 kg are estimated for the pre-atmospheric masses of the meteorites 77015 and 77260 respectively, based on the data on recovered

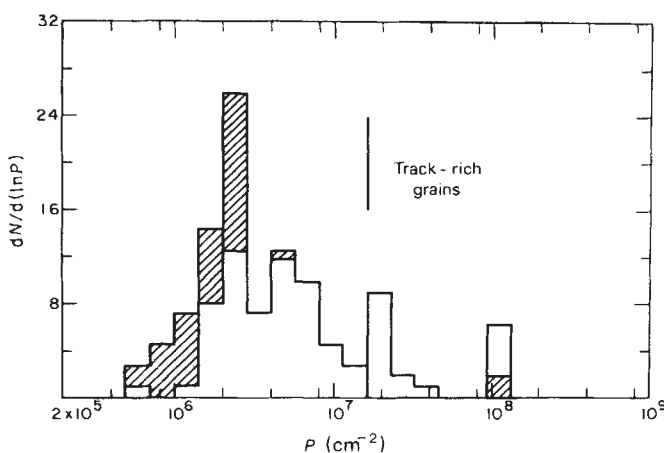
masses of these meteorites<sup>1</sup>, and the shielding depth estimates given in Table 1. The track data for these two samples do not rule out the possibility of these two samples belonging to the same fall as suggested recently<sup>6</sup> by their similar radionuclide concentrations. If both samples were originally parts of the same object, one would expect a slightly higher  $^{53}\text{Mn}$  concentration in the sample having higher shielding, which indeed is the case.

An important characteristic of the Antarctic meteorites, such as the H-3 chondrite 77003, is their long terrestrial residence times, generally in excess of  $10^4$  yr, as revealed by the  $^{14}\text{C}$  activities measured in these meteorites<sup>8</sup>. Thus they are distinctly different from the non-Antarctic meteorites, most of which are recent falls over the past 200 yr. It is therefore important to check whether the irradiation and evolutionary history of these two classes of meteorite show similar trends. The gas-rich meteorites of different types are ideal probes for such an investigation, and identification of additional gas-rich Antarctic meteorites belonging to different types is extremely important.

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**Note added in proof:** A recent study of noble gas contents in Antarctic H-chondrites revealed the presence of solar-type rare gases in the sample 75028 (N. Takaoka, personal communication).

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**Fig. 2** Track density frequency distribution for olivine and pyroxene grains from two specimens of the Antarctic meteorite 77216. The distribution is normalized to 100 grains.  $P$ , Number of tracks. □, Sample 77216, 18; ▨, Sample 77216, 24.  $N = 122$ .

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## Solidification zoning and metallographic cooling rates of chondrites

John Willis & Joseph I. Goldstein

Department of Metallurgy and Materials Engineering, Lehigh University, Bethlehem, Pennsylvania 18015, USA

The cooling rates of meteorites provide important clues to the sizes of their bodies. The cooling rates of chondritic meteorites have been determined by measuring the concentration of nickel in the interiors of taenite grains of various sizes and by comparing these data with computer models for the Ni distribution as a function of cooling rate<sup>1</sup>. It has been recently suggested<sup>2,3</sup> that in some meteorites the measured Ni gradients in taenite are not entirely produced by solid-state diffusion but partly reflect Ni gradients produced during solidification coring. If this is correct, Wood's cooling rate method<sup>1</sup> would be invalidated for these meteorites. We have investigated the effect of zoning produced during solidification on the formulation of the Wood model, and report here that solidification zoning is erased during kamacite growth and has no influence on the resultant taenite Ni gradients for all cases in which the cooling rate is sufficiently slow ( $<1,000\text{K Myr}^{-1}$ ) to allow Ni to diffuse to the taenite grain centre.

A metallographic study by Bevan and Axon<sup>2</sup> of the Tieschitz H3 chondrite revealed the presence of polycrystalline taenite in metallic spherules or chondrules, which they attributed to rapid solidification. They proposed<sup>2</sup> that the metal grains were compositionally zoned by rapid non-equilibrium solidification and that these gradients were retained by rapid cooling down to  $\sim 970\text{K}$ . During slow cooling below  $\sim 970\text{K}$ , kamacite growth

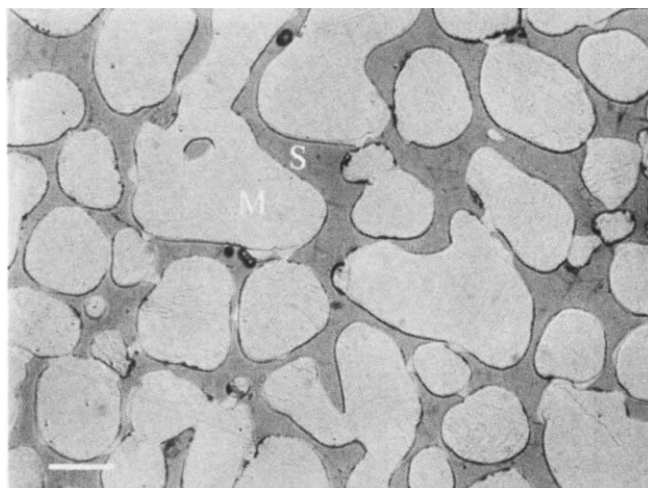


Fig. 2 Fe-Ni spheres (M) in an FeS matrix (S) formed by rapid cooling from a melt. The bulk composition of the melt was 9 wt% Ni, 8 wt% S. The average Ni composition of the spheres was 11.2%. Lightly etched with 2% nital. Scale bar, 10  $\mu\text{m}$ .

occurred and the Ni zoning produced at high temperatures was enhanced. Because the cooling rates determined by Wood<sup>1</sup> assume that metal grains have been homogenized above  $970\text{K}$ , Bevan and Axon<sup>2</sup> claim that the existence of prior zoning means that Wood's cooling rates underestimate the true cooling rate of the meteorite. They claim that the cooling rate for Tieschitz may be three orders of magnitude faster than Wood's value of  $1\text{K Myr}^{-1}$  (ref. 1). Furthermore, Hutchinson *et al.*<sup>3</sup> have argued that the presence of polycrystalline taenite in other more equilibrated H chondrites invalidates the application of the Wood technique in these cases and throws considerable doubt on its application to chondrites in general.

Scott and Rajan<sup>4</sup> suggest that the polycrystallinity of the taenite may be induced by hot working or that it may result from the accretion of small metal grains which failed to coarsen. They claim that even if the grains are initially zoned, cooling times necessary to produce kamacite particles of the dimensions found in the chondrites or to allow volume diffusion to alter gradients are sufficient to erase effectively the zoning produced during solidification. If this argument is correct, then the Wood cooling rate technique can still be applied.

To resolve this controversy, we have recently written a computer program<sup>5</sup> which is similar to that of Moren and Goldstein<sup>6</sup> in that it uses the Crank-Nicholson finite difference approximation to the diffusion equation and the Murray-Landis variable grid transformation for the interface movement. The major changes are that we use spherical rather than planar geometry and the Fe-Ni phase diagram of Romig and Goldstein<sup>7</sup>. Kamacite was assumed to form on the outer edge of a taenite sphere of uniform initial composition. The kamacite/taenite interface then moved radially inwards during cooling while the total metallic sphere diameter remained constant. This model produces cooling rate curves almost identical to those of Wood's model, in which kamacite and taenite particles exist as individual particles, either contiguous or separated by silicates, and Ni gradients in taenite are calculated as the taenite particle decreases in size. The cooling rate was assumed to decrease exponentially and the values quoted here are those at  $773\text{K}$ . Our results for particles with an initial uniform Ni composition of 10 wt% are shown in Fig. 1, with the curves of Wood<sup>1</sup>. Our curves are shifted towards higher Ni values and yield faster cooling rates by a factor of 2. Cooling rate curves for metal contents of 20 and 30 wt% are almost identical to those of 10 wt% Ni (ref. 5). Deviations occur when the Ni concentrations of the taenite approach that of the starting composition.

The initial conditions for the calculations were altered to incorporate a concentration gradient produced by solidification.

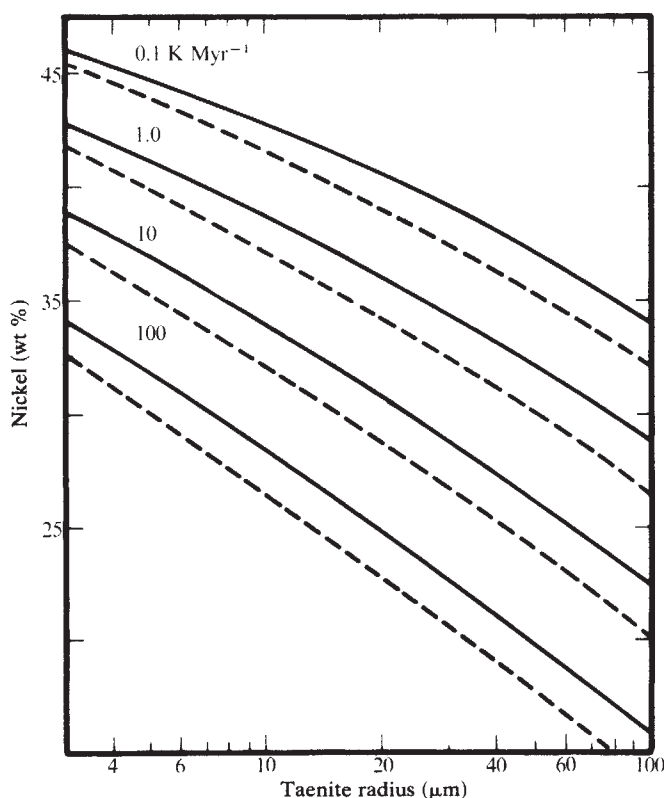
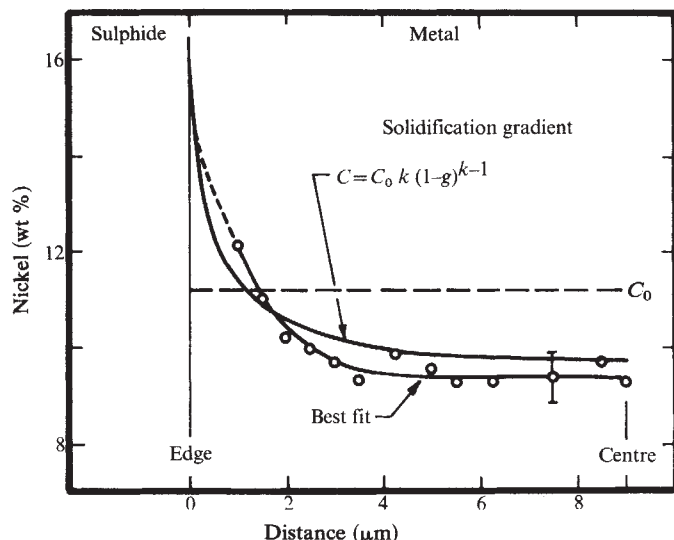


Fig. 1 Comparison of the cooling rate curves computed here (—) with those calculated by Wood<sup>1</sup> (---). Both sets of curves assume a uniform starting Ni content of 10 wt%. Our new curves yield cooling rates faster by a factor of 2.





**Fig. 3** Electron microprobe analysis across one of the spheres in the sample shown in Fig. 2. A smooth curve, labelled best fit, was drawn through the data and used as one of the starting conditions in the cooling rate calculations. Also shown is the Rayleigh equation for fractional crystallization and the Ni distribution that it predicts using values of 11.2% for  $C_0$ , the bulk concentration, and 0.87 for  $k$ , the distribution coefficient.

The Rayleigh equation<sup>8</sup> was used to calculate a theoretical solidification gradient and is the most extreme gradient that can be produced in this way. In addition, an Fe-Ni-S melt was prepared and quenched, producing a structure (Fig. 2) resembling that shown in Fig. 2c of Bevan and Axon<sup>2</sup>. The grain sizes seen in our melt and those found in Tieschitz are consistent with their being produced by solidification with a cooling rate of  $\sim 50\text{K s}^{-1}$ . A gradient produced during solidification was measured using the electron microprobe (Fig. 3); and it was also used as a starting profile in the computer model. The differences between this gradient and that of Rayleigh reflect the influence of diffusion after solidification and the inability of the microprobe to record steep gradients.

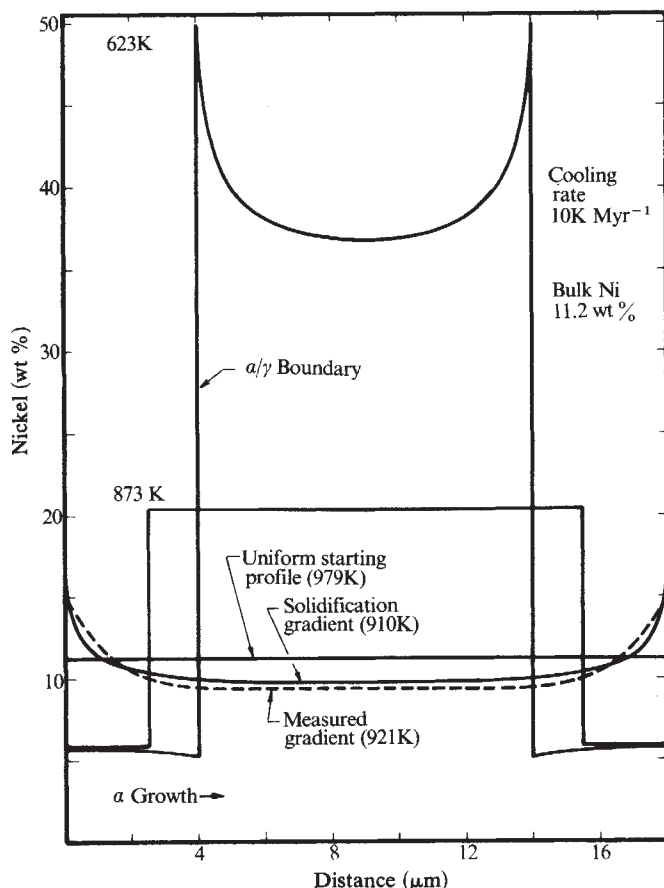
Cooling rate curves for 10% Ni computed using both a uniform initial taenite composition and a solidification zoned taenite are identical for 1, 10 and  $100\text{K Myr}^{-1}$ . Only at  $1,000\text{K Myr}^{-1}$  does a deviation occur. Prior zoning depresses the  $1,000\text{K Myr}^{-1}$  curve, for grains of radii  $> 20\text{ }\mu\text{m}$ , towards the starting Ni concentration of the grain centre (8.7%). Contrary to the claim of Bevan and Axon<sup>2</sup>, this has the effect of overestimating the cooling rates for chondrites in which a gradient has been established previously. Figure 4 illustrates the development of the Ni gradient for three starting conditions for an 18- $\mu\text{m}$  diameter grain cooling at  $10\text{K Myr}^{-1}$ . When the temperature reaches 870K, the three profiles become virtually identical. Similar sets of profiles were generated for the faster cooling rates, with similar results: by 870K, the profiles become indistinguishable. The major difference between the growth sequences occurs between 980 and 900K. As a result of the increase in Ni concentration at the grain boundaries during solidification, kamacite nucleation is suppressed by 50 to 70K.

Our calculations show that the gradients formed during solidification will be leveled during cooling for cooling rates less than  $1,000\text{K Myr}^{-1}$  and for particle radii  $< 100\text{ }\mu\text{m}$ . For cooling rates greater than  $1,000\text{K Myr}^{-1}$ , the portion of the curves for radii greater than  $20\text{ }\mu\text{m}$  will be depressed, so that cooling rates determined using the standard Wood curves will be overestimated.

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**Fig. 4** Starting, intermediate and final Ni profiles showing the gradient development for three different starting conditions. Due to the build-up of Ni at the taenite rim, the solidification profile and measured profile cases have suppressed nucleation temperatures. However, by 870K, all three starting conditions yield a similar, almost flat gradient.

## Peat bog records of atmospheric mercury deposition

Poul Pheiffer Madsen

Danish Isotope Centre/ATV, 2, Skelbækgade, DK 1717, Copenhagen V, Denmark

There have recently been many investigations using atmospheric mercury deposition as an indicator of the impact of industrial development, especially in the Northern Hemisphere. Thus, mosses have been used as indicators of airborne mercury pollution<sup>1,2</sup>, both wet and dry deposition have been analysed for mercury<sup>3,4</sup>, and mercury concentrations in  $^{18}\text{O}/^{16}\text{O}$  dated ice core samples from the Greenland Ice Sheet have been determined<sup>5</sup>. Heavy metal investigations of this kind from both Antarctica and Greenland are reviewed in ref. 6. I present here mercury fluxes calculated from measured concentrations in  $^{210}\text{Pb}$ -dated peat bog samples. The peat cores were taken from two widely separated ombrotrophic peat bogs in Denmark to elucidate the recent development in the atmospheric mercury deposition rate around the industrialized areas of Europe.

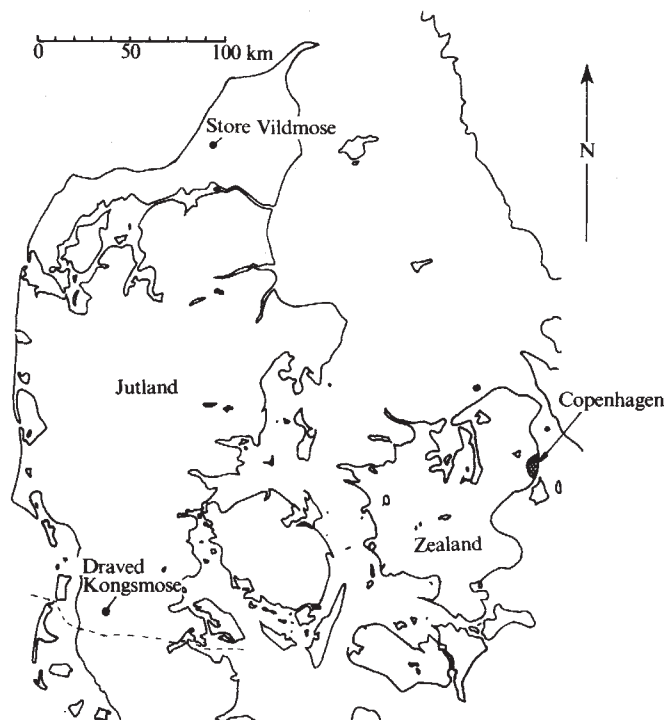


Fig. 1 Locations of peat bogs.

An ombrotrophic bog is an excellent source for such a study because the plants are dependent on the atmosphere for their nutrients and have evolved so that such inputs, including fall-out of heavy metals, are retained. There is clear evidence<sup>1,2,7</sup> that mercury is strongly retained by the humic substances of the peat even at the low pH of 3–4 characteristic of peat water which prevents the formation and escape of the water insoluble and volatile dimethylmercury. Beier and Jerneløv<sup>8</sup> have demonstrated that no dimethylmercury was formed in organic sediments with pH below 5. Furthermore, the sampled bogs contain areas where the peat has accumulated uninterrupted over hundreds of years.

The two bogs, Store Vildmose and Draved Kongsmose<sup>9,10</sup>, are situated in the northern and southern Jutland respectively (Fig. 1). Cores from the bogs were sampled by the Geological Survey of Denmark in the autumns of 1978 and 1979. These cores were frozen and then cut into slices 5–10 mm thick. Subsamples for dating were subsequently freeze-dried and subsamples for mercury determinations were kept frozen until analysis. The sampling procedure is described elsewhere<sup>10</sup>.

<sup>210</sup>Pb dating of the cores was performed by the Danish Isotope Centre according to the method described in ref. 11. The dating of the core from Draved Kongsmose was validated by <sup>14</sup>C dating and by comparison with measurements of the growth rates of the sphagnum mosses from whose remains the peat is formed<sup>11</sup>. Age versus depth curves are given in Fig. 2; standard deviations based on counting statistics are indicated.

Mercury was determined by flameless atomic absorption spectrometry. Some values were confirmed by neutron activation analysis and the method was validated by analysis of NBS standard reference material no. 1575, Pine Needle. The results, shown in Table 1, agree closely with those of Rühling and Tyler<sup>2</sup>, who found mercury contents in mosses from the Scandinavian Peninsula to be in the range 100–400 ng per g dry matter.

The mercury deposition rate for each subsample in the two cores has been calculated from the mercury concentration, the bulk density and the time of formation evaluated from the age versus depth curves. Curves of deposition rates versus age in Fig. 3 show the same general trends with considerable match in the detail. Minor discrepancies between the time scales can

Table 1 Variation of mercury concentrations with depth in ombrotrophic bogs Draved Kongsmose and Store Vildmose

| Depth below surface (cm) |                | Mercury concentration (ng per g) (dry matter) |                |
|--------------------------|----------------|-----------------------------------------------|----------------|
| Draved Kongsmose         | Store Vildmose | Draved Kongsmose                              | Store Vildmose |
| 0–1                      | 0–1.3          | 243                                           | 314            |
| 1–2                      | 1.3–2.4        | 77                                            | 331            |
| 2–3                      | 2.4–3.2        | 111                                           | 259            |
| 3–4                      | 3.2–4.8        | 134                                           | 287            |
| 4–5                      | 4.8–6.5        | 160                                           | 360            |
| 5–6                      | 6.5–7.5        | 176                                           | 382            |
| 6–7                      | 7.5–9.0        | 189                                           | 243            |
| 7–8                      | 9.0–9.9        | 202                                           | 228            |
| 8–9                      | 9.9–11.1       | 217                                           | 262            |
| 9–10                     | 11.1–12.0      | 201                                           | 243            |
| 19–11                    | 12.0–13.0      | 107                                           | 166            |
| 11–12                    | 13.0–14.2      | 189                                           | 210            |
| 12–13                    | 14.2–15.6      | 147                                           | 236            |
| 13–14                    | 15.6–16.9      | 402                                           | 151            |
| 14–15                    | 16.9–18.4      | 230                                           | 268            |
| 15–16                    | 18.4–19.6      | 151                                           | 741            |
| 16–17                    | 19.6–20.8      | 156                                           | 127            |
| 17–18                    | 20.8–22.2      | 387                                           | 138            |
| 18–19                    | 22.2–23.8      | 67                                            | 128            |
| 19–19.6                  | 23.8–25.4      | 74                                            | 150            |
| 19.6–20.5                | 25.4–27.2      | 190                                           | 137            |
| 20.5–21.5                | 27.2–28.8      | 258                                           | 227            |
| 21.5–22.5                | 28.8–29.6      | 380                                           | 523            |
| 22.5–23.3                | —              | 151                                           | —              |

probably be explained by the standard deviations of age indicated in Fig. 2.

It has been possible to validate some of the recent deposition rates from Draved Kongsmose by comparing them with results obtained by analysis of precipitation. Unfortunately this has not been possible for Store Vildmose as the subsamples cover too wide a span of years. Table 2 shows that the deposition rates obtained for 1976–77 agree very well with estimates based on

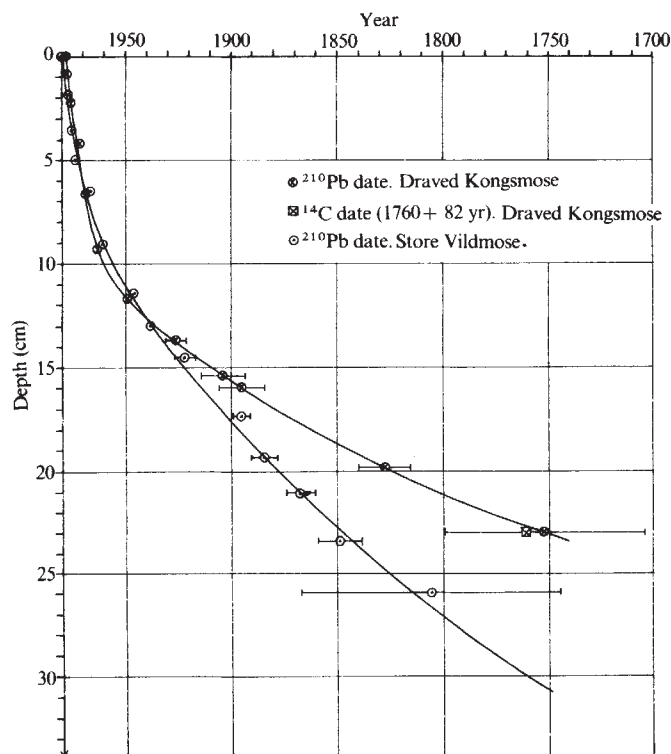
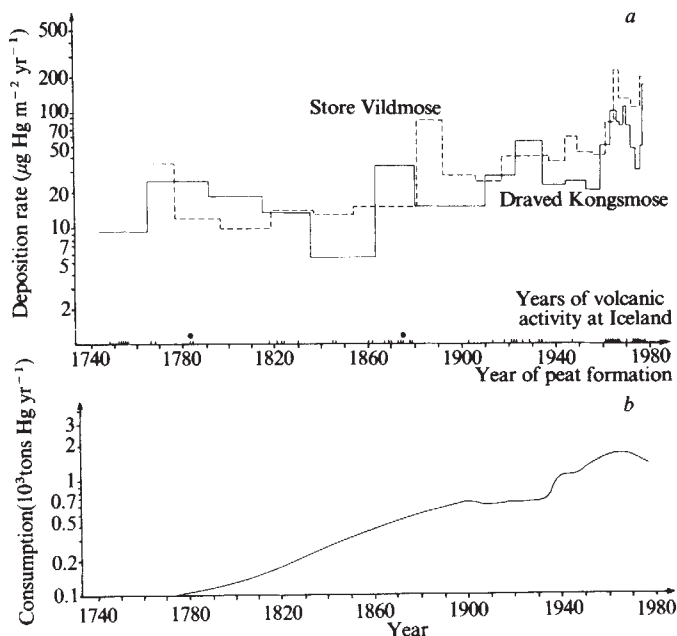


Fig. 2 Age versus depth for the ombrotrophic bogs Draved Kongsmose and Store Vildmose.





**Fig. 3** a, Atmospheric deposition rates of mercury in Draved Kongsmose and Store Vildmose. ●, Dust observed in Scandinavia. b, Development of consumption of mercury in the European Community countries.

analysis of precipitation collected monthly at Zealand (see Fig. 1) over the same period. This precipitation was analysed by low-level neutron activation analysis according to the method described by Jensen and Carlsen<sup>12</sup>. Between 1974 and 1976 the rates of mercury deposition to the peat at Draved Kongsmose were similar to that recorded in rainwater collected in Scotland during 1975 (ref. 13).

Based on the mercury concentration data of Appelquist *et al.*<sup>5</sup> and an average ice accumulation rate of  $259 \text{ mm yr}^{-1}$  (C. Hammer, personal communication) the mercury accumulation rate at site Crete at the Greenland Ice Sheet can be calculated to be  $3 \mu\text{g m}^{-2} \text{ yr}^{-1}$  for the periods 1772–74 and 1957–69, and  $1 \mu\text{g m}^{-2} \text{ yr}^{-1}$  for the period 1775–86. Thus the peat bog records show levels between one and two orders of magnitude higher.

The large fluctuations in deposition rates observed in both cores might be an artefact resulting from variations in growth dynamics or differences in metal binding capacity of the peat layers. However, Aaby *et al.*<sup>10</sup> analysed the Draved Kongsmose core for lead and found no such evidence. The two curves in Fig. 3, therefore, are considered to reflect the variations in the atmospheric mercury deposition rate during the past 200 years.

Both cores show a long-term increase in the rate since 1800–50. The Store Vildmose core, which is the northern one, seems to exhibit generally higher rates. Superimposed on the long-term trend several periods show elevated levels. Especially noticeable are peaks approximately during the periods 1760–

90, 1860–90, 1910–30, 1960–70 and 1973–78. Possible reasons for a long-term increase of this nature include increased circulation rate through natural changes, such as temperature elevation, increased precipitation and increased geothermal or volcanic activity. However, in Denmark the 30-yr average temperature has only increased by  $\sim 1^\circ\text{C}$  (13%) and the average rainfall has increased by only  $\sim 100 \text{ mm yr}^{-1}$  (18%) since the middle of the nineteenth century<sup>14</sup>. These changes are considered insufficient to cause an increase in mercury circulation rate of the magnitude shown in Fig. 3a. Neither is there evidence of continually increasing geothermal or volcanic activity during this period<sup>15</sup>. Thus the long-term increase is probably caused by the increased activity of man, directly or indirectly.

The development of the consumption of mercury in the EEC countries<sup>16</sup> is shown in Fig. 3b, as an indicator of human activity. The slope of this curve corresponds fairly well with the general trend in Fig. 3a. Reasons for the increasing mercury flux might be increasing mercury emissions from industrial use of mercury, burning of fuel, heating of ceramic materials, or increasing mercury degassing rate from agricultural soils. The latter may result from the general increase in agricultural area and/or intensified treatment and fertilization of soil. In fact the area carrying grains, beets and potatoes in Denmark has increased by a factor of 2–3 since 1860<sup>17</sup>. Furthermore, the increased use of lime to raise the pH of agricultural soil over the period being considered might have resulted in an enhanced formation of the volatile dimethylmercury in the soil.

From the global mass balance determined by Lantzy and MacKenzie<sup>18</sup>, an average atmospheric deposition rate of  $90 \mu\text{g m}^{-2} \text{ yr}^{-1}$  can be evaluated and attributed to degassing (42%), burning of fossil fuels (32%) and industrial sources (26%). However, whether this is valid for the localities of the actual peat bogs is uncertain.

With respect to the episodic short periods of elevated flux, the anthropogenic sources cannot be excluded for the periods 1960–70 and 1973–78 as there is some correlation with the patterns of mercury consumption in both EEC countries<sup>19</sup> and Denmark<sup>20</sup>. This seems a very unlikely cause of the 1760–90 and 1860–90 maxima although no reliable quantitative data on consumption are available for these periods. However, volcanic eruptions have been considered to be sources of atmospheric mercury<sup>21</sup>. As cyclones from Icelandic area often pass over the Scandinavian countries years of volcanic activity in Iceland are marked on Fig. 3a<sup>15,22</sup>. Periods with increased frequency of volcanic activity show generally increased levels of atmospheric mercury deposition. Three years in particular, should be noted because dust from eruptions was observed in Scandinavia<sup>15</sup> from the Laki eruption in 1783–84, the Askja eruption in 1875 and the Hekla eruption in 1947. The dust clouds from these eruptions are recorded to have passed Denmark to the north, which might explain why the Store Vildmose core shows the highest deposition rates. However, there might be some correlation between periods of low barometric pressure and the volcanic eruptions as suggested by Lamb<sup>23</sup>. A decrease in barometric pressure would also increase the degassing rate of mercury from the soil surface thereby increasing the amount washed out by rainfall.

It can be concluded, therefore, that the atmospheric mercury deposition rate in Denmark has shown a general increase over the past 100–200 yr, probably due to human activities, while periodically elevated rates might be caused by volcanic activity in Iceland or associated climatic events.

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**Table 2** Peat bog deposition rates of mercury compared with rates derived from analysis of precipitation

| Location                 | Period                       | Mean mercury deposition rate ( $\mu\text{g m}^{-2} \text{ yr}^{-1}$ ) | Ref.      |
|--------------------------|------------------------------|-----------------------------------------------------------------------|-----------|
| Zealand, Denmark         | October, 1976–September 1977 | 54                                                                    | 4*        |
| Copenhagen, Denmark      | October 1976–September 1977  | 65                                                                    | 4*        |
| Draved Kongsmose         | 1976–77                      | 53                                                                    | This work |
| Draved Kongsmose         | 1974–76                      | 31                                                                    | This work |
| Firth of Forth, Scotland | 1975                         | 27                                                                    | 11*       |

\* Based on concentration in precipitation and amount of rainfall.

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## Pleistocene sediments beneath the Ross Ice Shelf

Thomas B. Kellogg & Davida E. Kellogg

Department of Geological Sciences and Institute for Quaternary Studies, University of Maine at Orono, Orono, Maine 04469, USA

**We report here the results of new micropalaeontological studies of sediments collected from beneath the Ross Ice Shelf at RISP site J9 (82°22'S, 168°38'W). RISP sediments hold the key to understanding the history of the West Antarctic Ice Sheet, dynamics of marine-based ice sheets, sedimentation processes that operate beneath ice shelves and perhaps the early glacial history of Antarctica. RISP sediments were formerly assigned an age of late middle Miocene on the basis of their diatom flora. Our diatom analyses do not support this age assignment: diatoms in RISP cores are of mixed ages ranging from Miocene to late Quaternary, thus indicating that the sediments record a history of reworking, the most recent of which could have occurred no earlier than late Pleistocene. These results support our previous work with Ross Sea sediments but throw considerable doubt on a recent hypothesis concerning Miocene palaeogeographical evolution of the Ross Sea region.**

Previous studies of Ross Sea cores conducted in our laboratory defined two sedimentary units separated by a transition zone of well sorted sandy material<sup>1–3</sup>. The upper unit, called unit A, consisted of soft-to-soupy, diatom-rich, silty mud of Holocene age. Five radiocarbon dates supported this age assignment. The lower unit (unit B) was a compact, till-like sediment that contained a reworked diatom flora of mostly heavily silicified species that had undergone extensive breakage and had a mixture of stratigraphic ranges in the Miocene, Pliocene and Pleistocene. Breakage and mixing of diatoms probably occurred during basal sliding of a grounded ice sheet. Sediment and diatoms were reworked on the northern part of the Ross Sea continental shelf by former advances of grounded West Antarctic ice. Each advance of grounded ice mixed interglacial material, similar to unit A, into the underlying till left by the preceding ice advance. Advancing grounded ice created unit B by compacting the sediments and reworking and fracturing diatoms and other microfossils. The most recent episode of subglacial reworking occurred during the late Brunhes (0.0 to 0.69 Myr BP)<sup>4</sup>, because diatoms of McCollum's<sup>5</sup> *Coscinodiscus lentigenosus* zone were found throughout unit B. We predicted that material similar to unit B should also be present in cores from beneath the Ross Ice Shelf, but that unit A, which is deposited today in conditions that include relatively open water for part of the year, should be absent. We were rather sceptical, therefore, when Brady and Martin<sup>6</sup> and Webb et al.<sup>7</sup> reported a late middle Miocene age for the RISP sediments.

Eleven short gravity cores, 15–93 cm long, were collected at site J9 during the 1977–78 field season<sup>7</sup>; 47 additional cores, with lengths up to 122 cm, were collected in 1978–79<sup>8</sup>. Two

lithological units (not to be confused with Ross Sea units A and B) were observed in each core. The upper unit is 5–22 cm thick and consists of pebble-granule diatomaceous mud<sup>7,8</sup> which is light olive-grey (5Y 5/2) in colour. The lower unit, the entire thickness of which is unknown, was at the time of collection more compact than the soft-to-soupy upper unit<sup>7</sup>. Both units have similar compositions but the lower unit differs in colour, as it is olive grey (5Y 3/2)<sup>7,8</sup>, and contains greyish orange (10YR 7/4) or light olive-grey (5Y 5/2) sediment clasts of diatomaceous ooze. An age of late middle Miocene was proposed by Brady and others for both units<sup>6,9</sup> on the basis of stratigraphically important diatoms. Additional support for this age assignment comes from studies of pollen and spores<sup>6</sup> and silicoflagellates and ebridians<sup>11</sup>. Rare Radiolaria and Foraminifera were also reported<sup>8,12</sup>.

To investigate the adequacy of the proposed late middle Miocene age for the RISP sediments, cores 7 and 8, both of which were collected during the 1977–78 field season, were carefully sampled from the centre of each core (to avoid contamination) at various levels from top to bottom (Fig. 2). Diatom slides were prepared using standard methods<sup>13</sup> and were examined exhaustively to determine which species were present. The results were used to make stratigraphic inferences. Quantitative counts of diatoms on each slide were later made so as to compare RISP floral assemblages with previously studied diatom floras of sediments from the Ross Sea region. In discussing our results, we do not differentiate individual RISP samples or discuss separately the two units in RISP cores because we found the diatom flora to be similar in all samples.

Although we concur with Brady<sup>6</sup> that middle Miocene diatoms are present in the RISP sediments, we believe that these specimens are reworked because we also observed several species that are considerably younger (Fig. 1). A similar conclusion was reached independently by core describers, working under Dennis Cassidy at the Antarctic Core Facility, Florida State University (J. Hattner, personal communication). Younger species occur throughout both units in all RISP samples that we have analysed; their relative abundances are similar to those calculated for Recent Ross Sea sediments<sup>10</sup>, although they are usually less abundant than the Miocene-aged forms. The younger species are usually relatively small, delicate, weakly silicified forms that may easily be overlooked when mixed with the larger, more striking Miocene forms.

Stratigraphic ranges were established by McCollum<sup>5</sup> for a large number of diatom species that occur in DSDP cores from the Ross Sea and adjacent sub-Antarctic areas. He correlated the stratigraphic ranges with palaeomagnetic stratigraphy for the past 4.5 Myr, and established diatom zones with tentative ages extending back into the Lower Miocene. McCollum's stratigraphy<sup>5</sup> is recognized as a preliminary effort because it is based on only a few widely scattered DSDP cores; the older part has been revised<sup>14</sup>. However, because it is based, in part, on data from the Ross Sea, McCollum's stratigraphy remains state-of-the-art for this region. Our stratigraphic conclusions are, therefore, subject to revision, but we doubt that future refinements will significantly alter the stratigraphic conclusions presented here.

Figure 1 shows the stratigraphic ranges of diatoms we encountered in RISP cores, as well as those listed by Brady<sup>6</sup>, for all species for which stratigraphic information is available<sup>5,14,15</sup>. Some of the species in Fig. 1 were also reported by Brady<sup>6,9</sup>, but he and Webb<sup>8,16</sup> specifically stated that no species younger than Miocene occur in the RISP cores. If we assume that McCollum's<sup>5</sup> stratigraphic ranges for the Pliocene and Pleistocene are essentially correct, and neither we nor Brady<sup>6,9</sup>, or Schrader<sup>14</sup> doubt this, then we must conclude from Fig. 1 that Miocene, Pliocene, Pleistocene and even late Pleistocene diatom species are present throughout RISP cores 7 and 8. Because our examination of the cores at the Antarctic Core Facility in Tallahassee and our study of the core descriptions<sup>8</sup> show that these two cores are representative of the entire suite of RISP cores, this conclusion encompasses all RISP material. RISP



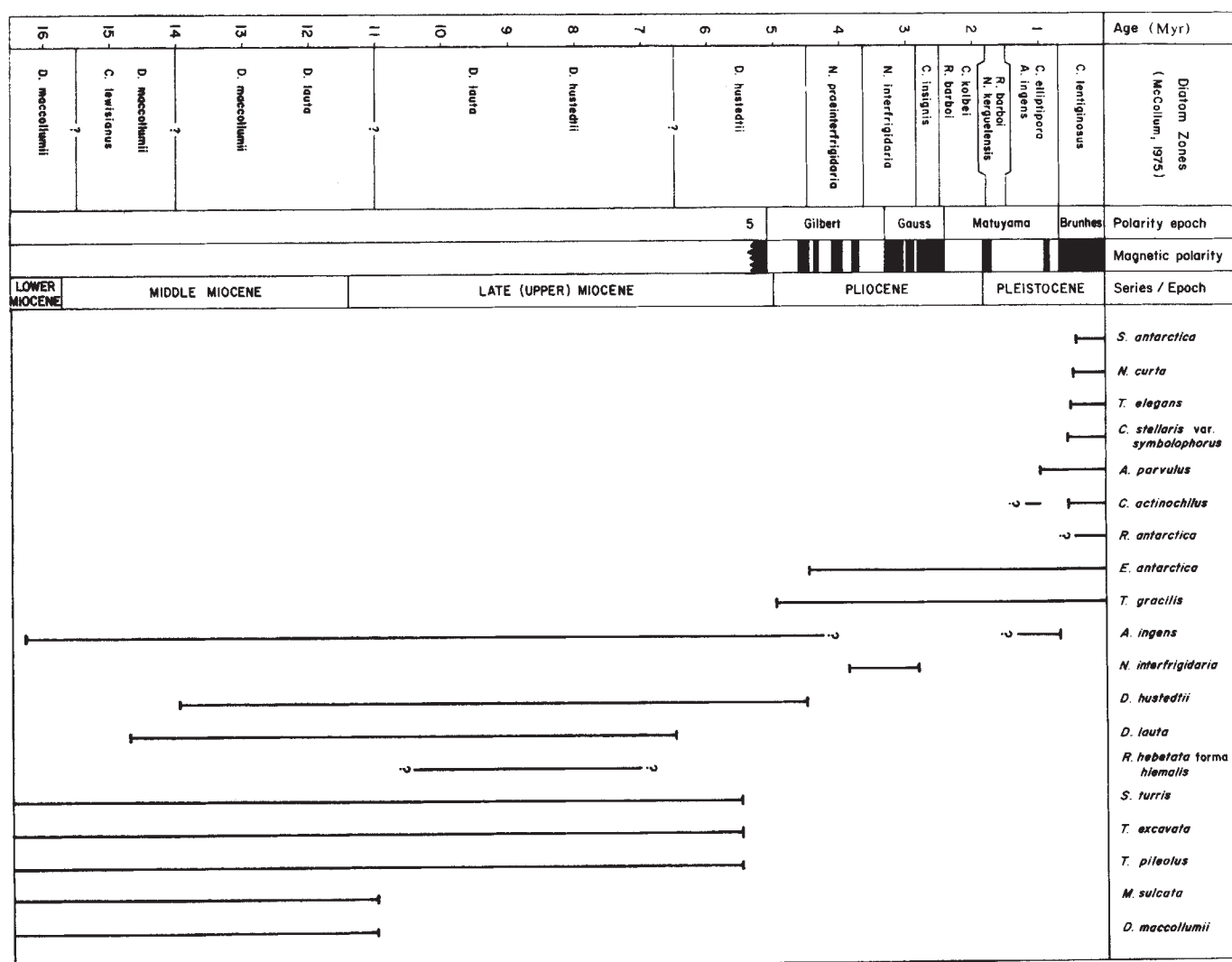
diatom assemblages are clearly reworked. We hypothesize that other Miocene microfossils and sediment in RISP cores are similarly reworked (as are Radiolaria, diatoms and Foraminifera in Ross Sea unit B<sup>1-3</sup>).

Our colleagues and several reviewers have suggested that our RISP samples might have been contaminated by recent diatoms in the water column that entered the cores during retrieval, or that we analysed samples of contaminated material from the edge of the cores. Neither suggestion is correct. Our samples were taken from the centres of the cores specifically to avoid edge contamination, and the material is so hard and compact<sup>8</sup> that contamination during retrieval is virtually impossible. Further, Pleistocene diatoms occur along with Miocene species in the sedimentary clasts of the lower RISP unit. We also took special care to avoid contamination in the laboratory. It is thus clear that the Pleistocene species we report are indeed present in the RISP samples. Our age interpretation is therefore as accurate as the currently known ranges for these species.

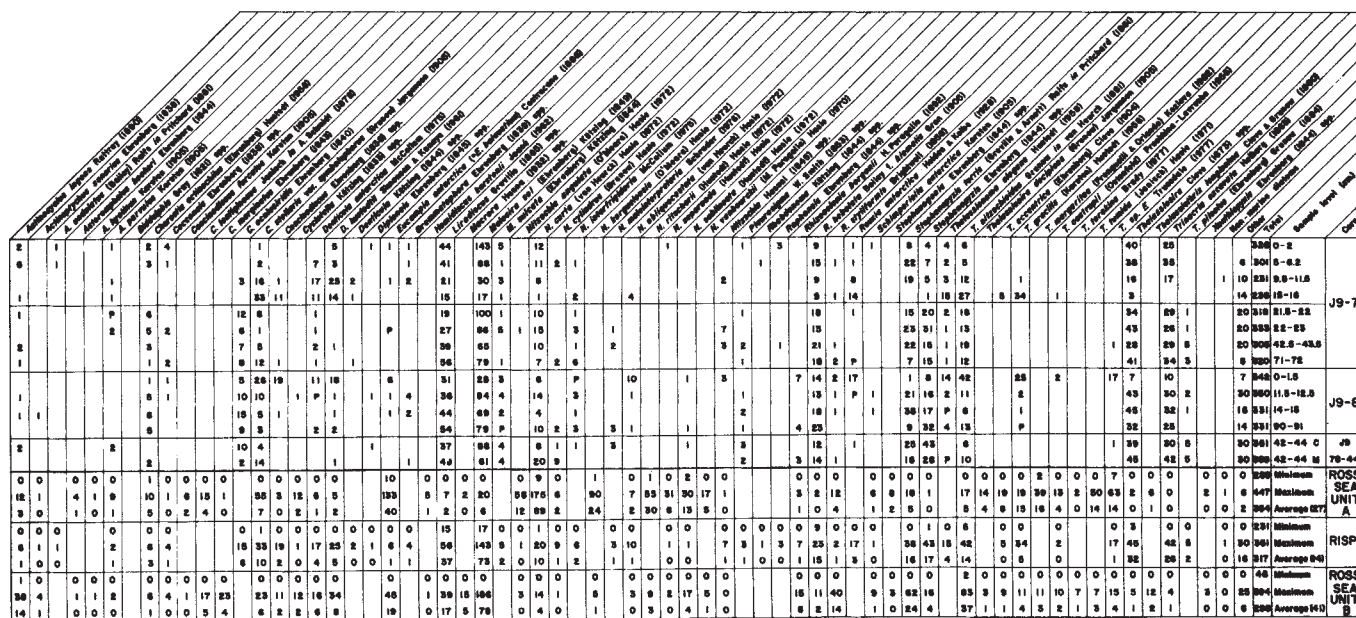
In addition to the diatom species listed in Fig. 1, we encountered several species throughout cores 7 and 8 for which no stratigraphic information is available for the Antarctic. We made quantitative counts of diatoms in each sample to obtain

data for comparison with diatom floras from Ross Sea cores. Figure 2 lists the numbers of specimens of all species that occurred during quantitative analyses (all species encountered were tallied during study of non-overlapping transects of each slide until ~300 specimens had been counted). As most of our slides contained many more than 300 specimens, it is not surprising that some of the rare Pleistocene species were not encountered during quantitative analyses. Also included in Fig. 2 are average counts for samples of units A and B from Ross Sea cores, and an indication of the variability involved in these data (minimum and maximum values).

Figure 2 shows a remarkable similarity between diatom floras in the RISP samples and in unit B. Nearly every species falls within the range of variability displayed in unit B<sup>2</sup>, but some differences are apparent: *Trinacria excavata* is considerably more abundant in the RISP samples than in any previously studied sample of unit B, *Eucampia antarctica* and species of the genus *Rhizosolenia* are less abundant in RISP material than they are in unit B, and *Coscinodiscus lentiginosus* was not observed in RISP samples. Diatom abundance and degree of preservation also appear nearly identical in samples from RISP site J9 and unit B.



**Fig. 1** Composite of stratigraphic ranges of diatoms in RISP sediment cores. Magnetic stratigraphy and series/epoch boundaries after Opdyke<sup>4</sup>. Diatom zones for the period from 4.5 Myr to the present are from McCollum<sup>5</sup>. Individual species' ranges are from McCollum<sup>5</sup>, except for *C. actinochilus* and *S. antarctica*<sup>14,15</sup>. *D. antarctica*, *T. excavata* and *T. pileolus* were reported from RISP cores by Brady<sup>9</sup>; *D. hustedtii*, *D. lauta*, *M. sulcata* and *R. hebetata* f. *hiemalis* were reported by Brady and Martin<sup>6</sup>.



**Fig. 2** Diatom census data for samples from RISP cores J9-7, J9-8 and J9 78-44. The boundary between the upper and lower sediment units occurs at 22 cm in J9-7, at 13 cm in J9-8 and at 17 cm in J9 78-44<sup>8</sup>. Samples from J9 78-44 at 42-44 cm represent analyses of a sedimentary clast (C) and sediment matrix from outside the clast (M). Comparative diatom census data, from ref. 2, are included for Ross Sea units A and B, with minimum and maximum tallies and averages of 27 and 41 sample analyses, respectively. Note that individual species' census data in RISP samples fall within the range of variability displayed in unit B. The letter 'P' is used to indicate that a species was present in a sample, but did not occur during quantitative analysis.

The absence of the Quaternary indicator species *C. lentiginosus* from RISP sediments may be merely an artefact of the very poor diatom preservation that characterizes these sediments. RISP sediments are a virtual hash of centric diatom fragments, which are too small for positive identification. Some of these fragments might well be broken specimens of *C. lentiginosus*, but positive identification of this large but delicate species rests largely on the presence of a marginal apiculus, which is often broken off even in relatively well preserved Ross Sea samples. The species that we can identify in RISP samples are those which are most likely to have survived intense mechanical reworking. It is this poor preservation of relatively delicate species which accounts for the observed predominance of robust Miocene diatoms like *Trinacria excavata* and *Stephanovxix turris* in RISP sediments.

The upper and lower units in RISP cores contain essentially identical diatom floras; these units differ only in colour and water content<sup>8</sup>. The lower unit in RISP cores is florally and sedimentologically the same as Ross Sea unit B, a basal till<sup>1-3</sup>. We conclude that the lower RISP unit is an extension of unit B and was formed by the same mechanism—reworking by grounded West Antarctic ice—as we hypothesized for unit B<sup>1-3</sup>.

The upper unit in cores from site J9 is problematic. Although it has the same floral assemblage and sedimentology as unit B, its high water content and lack of overcompaction argue against former deposition beneath grounded ice. We do not favour an explanation based on postglacial deposition beneath the Ross Ice Shelf. Even though modern diatoms are carried beneath the shelf by tidal pumping and ocean currents, the high degree of breakage of the diatoms and the fact that the assemblage in both units contains diatoms of mixed age suggest intense mechanical reworking. There is no evidence for strong bottom currents beneath the ice shelf<sup>17</sup>. We thus conclude that the upper unit in the RISF cores results from postglacial alteration of the basal till that forms the lower unit (unit B). The absence of similar material from the Ross Sea continental shelf north of the Ross Ice Shelf may be explained by the cover of Recent sediments (unit A) which prevents direct contact of the till with seawater, or it may be that some peculiar process operates beneath the ice shelf that has not yet been documented.

The microfossils must not be misinterpreted as *in situ*. We do not suggest that RISP diatoms are *in situ* Pleistocene deposits and believe we have documented that they are not *in situ* late middle Miocene. They are clearly reworked, and probably were reworked several times, the most recent episode of reworking having occurred during the late Brunhes.

Our analyses of the two RISP cores support the conclusions of our previous work<sup>1-3</sup>. The lower unit in the RISP cores seems to represent an extension of unit B. Unit A is not present in the RISP cores, as we predicted. In contrast to our expectations, the transition zone that occurs in cores from the open Ross Sea was not observed in the RISP cores. We hypothesized<sup>1,3</sup> that sediments in this zone were winnowed and selectively sorted by bottom currents associated with the grounding line of the retreating West Antarctic Ice Sheet. The currents were probably caused by a combination of subglacial meltwater streaming from beneath the ice sheet, marine bottom water flow and tidal pumping. The absence of transition zone sediments at site J9 suggests that the grounding line was retreating rapidly at the time it passed this area.

We conclude that the RISP sediments and unit B represent material that was reworked and compacted by grounded West Antarctic ice. The most recent reworking, which occurred during the late Brunhes, probably represents an advance of the West Antarctic Ice Sheet during late Wisconsin time. Precise dating of this event in Ross Sea and RISP sediments has not yet been accomplished, but glacial geological studies in the McMurdo Sound region and along the coast of northern Victoria Land support a late Wisconsin age<sup>18,19</sup>.

The significance of our results extends beyond merely confirming our previous work with Ross Sea sediments. The presence of late Pleistocene material throughout the sediment cores from site J9, in contrast to previously published RISP results<sup>6-9,16,20-22</sup>, suggests that future acquisition of longer cores from beneath the Ross Ice Shelf may provide information on the history of Quaternary and Pliocene fluctuations of the West Antarctic Ice Sheet. In addition, it throws considerable doubt on a recent hypothesis concerning Miocene palaeogeographical evolution of the Ross Sea region<sup>7,22</sup> as this hypothesis requires a middle Miocene age for RISP sediments.



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## A computer algorithm for reconstructing a scene from two projections

H. C. Longuet-Higgins

Laboratory of Experimental Psychology, University of Sussex, Brighton BN1 9QG, UK

A simple algorithm for computing the three-dimensional structure of a scene from a correlated pair of perspective projections is described here, when the spatial relationship between the two projections is unknown. This problem is relevant not only to photographic surveying<sup>1</sup> but also to binocular vision<sup>2</sup>, where the non-visual information available to the observer about the orientation and focal length of each eye is much less accurate than the optical information supplied by the retinal images themselves. The problem also arises in monocular perception of motion<sup>3</sup>, where the two projections represent views which are separated in time as well as space. As Marr and Poggio<sup>4</sup> have noted, the fusing of two images to produce a three-dimensional percept involves two distinct processes: the establishment of a 1:1 correspondence between image points in the two views—the ‘correspondence problem’—and the use of the associated disparities for determining the distances of visible elements in the scene. I shall assume that the correspondence problem has been solved; the problem of reconstructing the scene then reduces to that of finding the relative orientation of the two viewpoints.

Photogrammetrists know that if a scene is photographed from two viewpoints, then the relationship between the camera positions is uniquely determined, in general, by the photographic coordinates of just five distinguishable points; but actually calculating the structure of the scene from five sets of image coordinates involves the iterative solution of five simultaneous third-order equations<sup>1</sup>. I show here that if the scene contains as many as eight points whose images can be located in each projection, then the relative orientation of the two projections, and the structure of the scene, can be computed, in general, from the eight sets of image coordinates by a direct method which

calls for nothing more difficult than the solution of a set of simultaneous linear equations.

Let  $P$  be a visible point in the scene, and let  $(X_1, X_2, X_3)$  and  $(X'_1, X'_2, X'_3)$  be its three-dimensional cartesian coordinates with respect to the two viewpoints. The ‘forward’ coordinates  $X_3$  and  $X'_3$  are necessarily positive. The image coordinates of  $P$  in the two views may then be defined as

$$\begin{aligned} (x_1, x_2) &= (X_1/X_3, X_2/X_3), \\ (x'_1, x'_2) &= (X'_1/X'_3, X'_2/X'_3) \end{aligned} \quad (1)$$

and it is convenient to supplement them with the dummy coordinates

$$x_3 = 1, \quad x'_3 = 1 \quad (2)$$

so that one can then write

$$x_\mu = X_\mu/X_3, \quad x'_\nu = X'_\nu/X'_3 \quad (\mu, \nu = 1, 2, 3) \quad (3)$$

As the two sets of three-dimensional coordinates are connected by an arbitrary displacement, we may write

$$X'_\mu = \mathbf{R}_{\mu\nu}(X_\nu - \mathbf{T}_\nu) \quad (4)$$

where  $\mathbf{T}$  is an unknown translational vector and  $\mathbf{R}$  is an unknown rigid rotation matrix. (In this and subsequent equations I sum over repeated Greek subscripts.) The rotation  $\mathbf{R}$  satisfies the relationships

$$\mathbf{R}\tilde{\mathbf{R}} = \mathbf{1} = \tilde{\mathbf{R}}\mathbf{R}, \quad \det \mathbf{R} = 1 \quad (5)$$

and it is convenient to adopt the length of the vector  $\mathbf{T}$  as the unit of distance:

$$\mathbf{T}_\nu^2 (= \mathbf{T}_1^2 + \mathbf{T}_2^2 + \mathbf{T}_3^2) = 1 \quad (6)$$

I begin by establishing a general relationship between the two sets of image coordinates—a relationship which expresses the condition that corresponding rays through the two centres of projection must intersect in space. We define a new matrix  $\mathbf{Q}$  by

$$\mathbf{Q} = \mathbf{R}\mathbf{S} \quad (7)$$

where  $\mathbf{S}$  is the skew-symmetric matrix

$$\mathbf{S} = \begin{bmatrix} 0 & \mathbf{T}_3 & -\mathbf{T}_2 \\ -\mathbf{T}_3 & 0 & \mathbf{T}_1 \\ \mathbf{T}_2 & -\mathbf{T}_1 & 0 \end{bmatrix} \quad (8)$$

Equation (8) may be written as

$$\mathbf{S}_{\lambda\nu} = \varepsilon_{\lambda\nu\sigma} \mathbf{T}_\sigma \quad (9)$$

where  $\varepsilon_{\lambda\nu\sigma} = 0$  unless  $(\lambda, \nu, \sigma)$  is a permutation of  $(1, 2, 3)$ , in which case  $\varepsilon_{\lambda\nu\sigma} = \pm 1$  depending on whether this permutation is even or odd. It follows from equations (4)–(9) that

$$\begin{aligned} \mathbf{X}'_\mu \mathbf{Q}_{\mu\nu} \mathbf{X}_\nu &= \mathbf{R}_{\mu\kappa} (\mathbf{X}_\kappa - \mathbf{T}_\kappa) \mathbf{R}_{\mu\lambda} \varepsilon_{\lambda\nu\sigma} \mathbf{T}_\sigma \mathbf{X}_\nu \\ &= (\mathbf{X}_\lambda - \mathbf{T}_\lambda) \varepsilon_{\lambda\nu\sigma} \mathbf{T}_\sigma \mathbf{X}_\nu \end{aligned} \quad (10)$$

but because the quantity  $\varepsilon_{\lambda\nu\sigma}$  is antisymmetric in every pair of its subscripts, the right-hand side vanishes identically:

$$\mathbf{X}'_\mu \mathbf{Q}_{\mu\nu} \mathbf{X}_\nu = 0 \quad (11)$$

Dividing equation (11) by  $X'_3 X_3$  we arrive at the desired relationship between the image coordinates:

$$x'_\mu \mathbf{Q}_{\mu\nu} x_\nu = 0 \quad (12)$$

The next step is to determine the nine elements  $\mathbf{Q}_{\mu\nu}$ . There will be one equation of type (12) for every point  $P_i$ , namely

$$(x'_\mu x_\nu)_i \mathbf{Q}_{\mu\nu} = 0 \quad (13)$$

and in this equation the nine quantities  $(x'_\mu x_\nu)_i$  are presumed to be known. The ratios of the nine unknowns  $\mathbf{Q}_{\mu\nu}$  can therefore be obtained, in general, by solving eight simultaneous linear equations of type (13), one for each of eight visible points  $P_1, \dots, P_8$ . I shall not yet discuss the special circumstances under which the solution fails; for the present merely note that if the eight equations (13) are independent, their solution is entirely straightforward from a computational point of view.

The translational vector  $\mathbf{T}$  must be calculated next. Multiplying  $\mathbf{Q}$  on the left of equation (7) by its transpose we obtain

$$\tilde{\mathbf{Q}}\mathbf{Q} = \tilde{\mathbf{S}}\mathbf{R}\mathbf{R}\mathbf{S} = \tilde{\mathbf{S}}\mathbf{S} \quad (14)$$

so that, by the definition of  $\mathbf{S}$ ,

$$\mathbf{Q}_{\mu\nu}\mathbf{Q}_{\mu\sigma} = \mathbf{T}_{\mu}^2\delta_{\nu\sigma} - \mathbf{T}_{\nu}\mathbf{T}_{\sigma} \quad (15)$$

But  $\mathbf{T}_{\mu}^2 = 1$  by equation (6), and so the trace of  $\tilde{\mathbf{Q}}\mathbf{Q}$  must be

$$\mathbf{Q}_{\mu\nu}\mathbf{Q}_{\mu\nu} = \delta_{\nu\nu} - \mathbf{T}_{\nu}^2 = 2 \quad (16)$$

The nine elements of  $\mathbf{Q}$  can therefore be normalized by dividing them by  $\sqrt{\frac{1}{2}\text{trace } \tilde{\mathbf{Q}}\mathbf{Q}}$ ; the elements of the normalized matrix  $\tilde{\mathbf{Q}}\mathbf{Q}$  can then be used for computing the ratios of the components of  $\mathbf{T}$ :

$$\tilde{\mathbf{Q}}\mathbf{Q} = \begin{bmatrix} 1 - \mathbf{T}_1^2 & -\mathbf{T}_1\mathbf{T}_2 & -\mathbf{T}_1\mathbf{T}_3 \\ -\mathbf{T}_2\mathbf{T}_1 & 1 - \mathbf{T}_2^2 & -\mathbf{T}_2\mathbf{T}_3 \\ -\mathbf{T}_3\mathbf{T}_1 & -\mathbf{T}_3\mathbf{T}_2 & 1 - \mathbf{T}_3^2 \end{bmatrix} \quad (17)$$

There are evidently three independent relationships between the diagonal and the off-diagonal elements of  $\tilde{\mathbf{Q}}\mathbf{Q}$ ; these supply three independent checks on the results obtained so far. The absolute signs of the  $\mathbf{T}_{\mu}$  and the  $\mathbf{Q}_{\mu\nu}$  are still undetermined but, as we shall see, these ambiguities are easily resolved later.

We are now in a position to compute the elements of the rotation matrix  $\mathbf{R}$ . First note that equation (7) has a simple interpretation in terms of vector products. If we regard each row of  $\mathbf{Q}$ , and each row of  $\mathbf{R}$ , as a vector, then

$$\mathbf{Q}_{\alpha} = \mathbf{T} \times \mathbf{R}_{\alpha} \quad (\alpha = 1, 2, 3) \quad (18)$$

and the condition for  $\mathbf{R}$  to represent a proper rotation can be expressed in a similar form:

$$\mathbf{R}_{\alpha} = \mathbf{R}_{\beta} \times \mathbf{R}_{\gamma} \quad (19)$$

for  $\alpha, \beta, \gamma$  such that  $\epsilon_{\alpha\beta\gamma} = 1$ . The problem is then to express the  $\mathbf{R}_{\alpha}$  in terms of  $\mathbf{T}$  and the  $\mathbf{Q}_{\alpha}$ .

By equation (18),  $\mathbf{R}_{\alpha}$  is orthogonal to  $\mathbf{Q}_{\alpha}$  and may therefore be expressed as a linear combination of  $\mathbf{T}$  and  $\mathbf{Q}_{\alpha} \times \mathbf{T}$ . We therefore introduce new vectors

$$\mathbf{W}_{\alpha} = \mathbf{Q}_{\alpha} \times \mathbf{T} \quad (\alpha = 1, 2, 3) \quad (20)$$

and write

$$\mathbf{R}_{\alpha} = a_{\alpha}\mathbf{T} + b_{\alpha}\mathbf{W}_{\alpha} \quad (21)$$

Substitution into equation (18) gives

$$\mathbf{Q}_{\alpha} = \mathbf{T} \times (a_{\alpha}\mathbf{T} + b_{\alpha}\mathbf{W}_{\alpha}) = b_{\alpha}(\mathbf{T} \times \mathbf{W}_{\alpha}) \quad (22)$$

But as  $\mathbf{T}$  is a unit vector,

$$\mathbf{T} \times \mathbf{W}_{\alpha} = \mathbf{T} \times (\mathbf{Q}_{\alpha} \times \mathbf{T}) = \mathbf{Q}_{\alpha} \quad (23)$$

and so

$$b_{\alpha} = 1 \quad (24)$$

Turning to equation (19) we deduce that when  $\epsilon_{\alpha\beta\gamma} = 1$ ,

$$\begin{aligned} a_{\alpha}\mathbf{T} + \mathbf{W}_{\alpha} &= (a_{\beta}\mathbf{T} + \mathbf{W}_{\beta}) \times (a_{\gamma}\mathbf{T} + \mathbf{W}_{\gamma}) \\ &= a_{\beta}\mathbf{Q}_{\gamma} - a_{\gamma}\mathbf{Q}_{\beta} + \mathbf{W}_{\beta} \times \mathbf{W}_{\gamma} \end{aligned} \quad (25)$$

But in equation (25) the vectors  $\mathbf{W}_{\alpha}$ ,  $\mathbf{Q}_{\beta}$  and  $\mathbf{Q}_{\gamma}$  are all orthogonal to  $\mathbf{T}$ , whereas  $\mathbf{W}_{\beta} \times \mathbf{W}_{\gamma}$  is, by equation (20), a multiple of  $\mathbf{T}$ . It follows that in equation (25) the first term on the left equals the last term on the right,

$$a_{\alpha}\mathbf{T} = \mathbf{W}_{\beta} \times \mathbf{W}_{\gamma} \quad (26)$$

and equation (21) finally becomes

$$\mathbf{R}_{\alpha} = \mathbf{W}_{\alpha} + \mathbf{W}_{\beta} \times \mathbf{W}_{\gamma} \quad (27)$$

Having obtained in this way the vector  $\mathbf{T}$  and the three rows of the matrix  $\mathbf{R}$ , we can at last find the three-dimensional coordinates  $\mathbf{X}_{\mu}$ , as follows:

By equation (4),

$$\mathbf{X}'_{\mu} = \mathbf{R}_{\mu\nu}(\mathbf{X}_{\nu} - \mathbf{T}_{\nu})$$

from which it follows that

$$\mathbf{x}'_1 = \frac{\mathbf{X}'_1}{\mathbf{X}'_3} = \frac{\mathbf{R}_{1\nu}(\mathbf{X}_{\nu} - \mathbf{T}_{\nu})}{\mathbf{R}_{3\nu}(\mathbf{X}_{\nu} - \mathbf{T}_{\nu})} \quad (28)$$

Introducing the vectors

$$\mathbf{X} = (\mathbf{X}_1, \mathbf{X}_2, \mathbf{X}_3), \quad \mathbf{x} = (\mathbf{x}_1, \mathbf{x}_2, 1) \quad (29)$$

we may write equation (28) in terms of the rows  $\mathbf{R}_{\alpha}$  of the matrix  $\mathbf{R}$ :

$$\mathbf{x}'_1 = \frac{\mathbf{R}_1 \cdot (\mathbf{X} - \mathbf{T})}{\mathbf{R}_3 \cdot (\mathbf{X} - \mathbf{T})} = \frac{\mathbf{R}_1 \cdot (\mathbf{x} - \mathbf{T}/\mathbf{X}_3)}{\mathbf{R}_3 \cdot (\mathbf{x} - \mathbf{T}/\mathbf{X}_3)} \quad (30)$$

from which it follows that

$$\mathbf{X}_3 = \frac{(\mathbf{R}_1 - \mathbf{x}'_1\mathbf{R}_3) \cdot \mathbf{T}}{(\mathbf{R}_1 - \mathbf{x}'_1\mathbf{R}_3) \cdot \mathbf{x}} \quad (31)$$

The other unprimed coordinates are then given by equation (3) as

$$\mathbf{X}_1 = \mathbf{x}_1\mathbf{X}_3, \quad \mathbf{X}_2 = \mathbf{x}_2\mathbf{X}_3 \quad (32)$$

and the primed coordinates are finally obtained from equation (4).

There are, in fact, four distinct solutions to the problem, associated with the alternative choices of sign for the components of  $\mathbf{T}$  and the elements of  $\mathbf{Q}$ . But any doubt as to which choices to adopt is easily resolved: the condition that the forward coordinates of any point must both be positive will be satisfied if, and only if, both sets of signs are correctly chosen.

There are certain 'degenerate' eight-point configurations for which the algorithm fails because the associated equations (13) become non-independent. A configuration will be degenerate if as many as four of the points lie in a straight line, or if as many as seven of them lie in a plane. Quite unexpectedly, degeneracy also arises if the configuration includes six points at the vertices of a regular hexagon, or consists of eight points at the vertices of a cube. The 'invisibility' of such configurations to the eight-point algorithm may be demonstrated by arguments too long to be presented here; but the reasons for it are unconnected with any ambiguity in the interpretation of the resulting projections. A degenerate configuration immediately becomes 'visible', however, if one of the offending points  $P_i$  is moved slightly away from its original position.

In general, then, the three-dimensional coordinates of a set of eight or more visible points may be obtained by the following algorithm:

- (1) Set up eight equations of the form (13), and solve them for the ratios of the nine unknowns  $\mathbf{Q}_{\mu\nu}$ .
- (2) Compute the matrix  $\tilde{\mathbf{Q}}\mathbf{Q}$  and normalize the elements of  $\mathbf{Q}$  by dividing them by  $\sqrt{\frac{1}{2}\text{trace } \tilde{\mathbf{Q}}\mathbf{Q}}$ .
- (3) Obtain the magnitudes and the relative signs of the  $\mathbf{T}_{\nu}$  from equation (17); their absolute signs, and those of the  $\mathbf{Q}_{\mu\nu}$ , may have to be chosen arbitrarily at this stage.
- (4) Define three new vectors by equation (20) and use equation (27) to calculate the rows of the matrix  $\mathbf{R}$ .
- (5) Use equations (31) and (32) for computing the unprimed three-dimensional coordinates of all the visible points, and equation (4) for calculating the primed coordinates.
- (6) Check that the forward coordinates  $\mathbf{X}_3$  and  $\mathbf{X}'_3$  of any point are both positive. If both signs are negative, alter the signs of the  $\mathbf{T}_{\nu}$  and return to step (5); if  $\mathbf{X}_3$  and  $\mathbf{X}'_3$  are of opposite sign, reverse the signs of the  $\mathbf{Q}_{\mu\nu}$  and return to step 4.

The algorithm yields the most accurate results when applied to situations in which the distance  $D$  between the centres of projection is not too small compared with their distances from the points  $P_i$ . If the projective coordinates are accurate to a few seconds of arc, the forward coordinates of the  $P_i$  can be estimated out to about  $10D$  with great accuracy, and even as far as  $100D$  if the  $P_i$  are adequately spaced in depth. This performance is comparable with that of the human visual system; but that does not, of course, imply that the eight-point algorithm is actually used in stereoscopic vision, as in binocular vision we



have at least some information about the relative orientation of the two eyes. The most useful applications of the eight-point algorithm will probably be found in computer vision systems, where there is still a need for fast and accurate methods of converting two-dimensional images into three-dimensional interpretations.

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## Local extinction and ecological re-entry of early Eocene mammals

David M. Schankler

Department of Geological and Geophysical Sciences,  
Princeton University, Princeton, New Jersey, USA

The use of high-resolution stratigraphical control in studying the species sequencing patterns of early Eocene mammals has recently been demonstrated by Gingerich *et al.*<sup>1–5</sup>. These studies have documented the nature of the tempo and mode of evolution and have stimulated the debate between adherents of phyletic gradualism and punctuated equilibria<sup>6</sup>. All studies have been largely verticalist (evolutionary) in outlook, equating change within a single basin of deposition with evolution. However, when I applied these techniques to the early Eocene condylarth, *Phenacodus*, I found a strong lateral (biogeographical and ecological) component influencing the sequencing of species in a single basin. I report here that *Phenacodus* species are relatively static in size and morphology throughout the local section studied, and two of these show statistically significant discontinuities in temporal range. The clumped re-entry of these species after local disappearance along with the introduction of other new taxa points to ecological control of vertical events within a local section.

I used high-resolution stratigraphy to study mammalian evolution at the species level, concentrating on early Eocene Willwood Formation of northwestern Wyoming because of the density and continuity of the fossil record preserved in these sediments. The Elk Creek Section through the Willwood

Formation of the central Bighorn Basin is 773 m thick<sup>7</sup>, and represents an estimated 3.5 Myr (ref. 8). Using a 10-m resolution interval, over 240 fossil localities can be related to this section. These localities, distributed over a 650-km<sup>2</sup> area, so far have produced over 15,000 specimens and are distributed vertically through the section so that more than 50 of the 77 10-m intervals are fossiliferous; half of these have total mammalian sample sizes (minimum number of individuals) of over 75.

In a recent review of the systematics of the genus *Phenacodus*, West<sup>9</sup> recognized three species from the early Eocene, *P. brachypternus*, *P. vortmani* and *P. primaevus*; his study predates publication of the Elk Creek Section and the stratigraphical resolution used was at the level of the zone, that is, early Eocene *Phenacodus* were divided into three samples corresponding to Graybullian (base–530 m), Lysitian (530–660 m) and Lostcabinian (660–770 m) zones. *P. brachypternus* was recorded as being almost wholly restricted to the Graybullian, whereas the other two species were distributed through the entire Wasatchian. *P. brachypternus* and *P. vortmani* are relatively well defined fossil species that have always been easily identified in collections on the basis of size and morphology, whereas *P. primaevus* has usually been characterized by a fairly large variation that has made it difficult to define the bounds of the species. This has led to the subdivision of *P. primaevus* into two to four species<sup>10,11</sup>. West<sup>9</sup> did not subdivide this species even though the recorded ranges of variation (coefficients of variation of ~10 for most linear measures) and a range of ln 0.8 for tooth crown area of M<sub>2</sub> (lower second molar) are almost double those expected for a single species<sup>12–14</sup>.

Inclusion of all large specimens of *Phenacodus* in a single species is untenable, and on the basis of data shown in Fig. 1, the large phenacodontids have been subdivided into two species, *P. intermedius* and *P. primaevus*. As previously shown by Gingerich<sup>12,13</sup> and observed in the two smaller species of *Phenacodus*, the normal range of variation of ln of the crown area of a molar tooth is of the order of 0.4. In the interval 100–190 m, almost all specimens fall neatly within the expected range of a single species, the size of which agrees well with the type of *P. intermedius*; thus these specimens are referred to that species. The single outlier at the 100-m level has a value that differs by 4 standard deviations from the mean of *P. intermedius* specimens, and is therefore too large to be included in that species. In the interval 200–380 m there is a second well defined

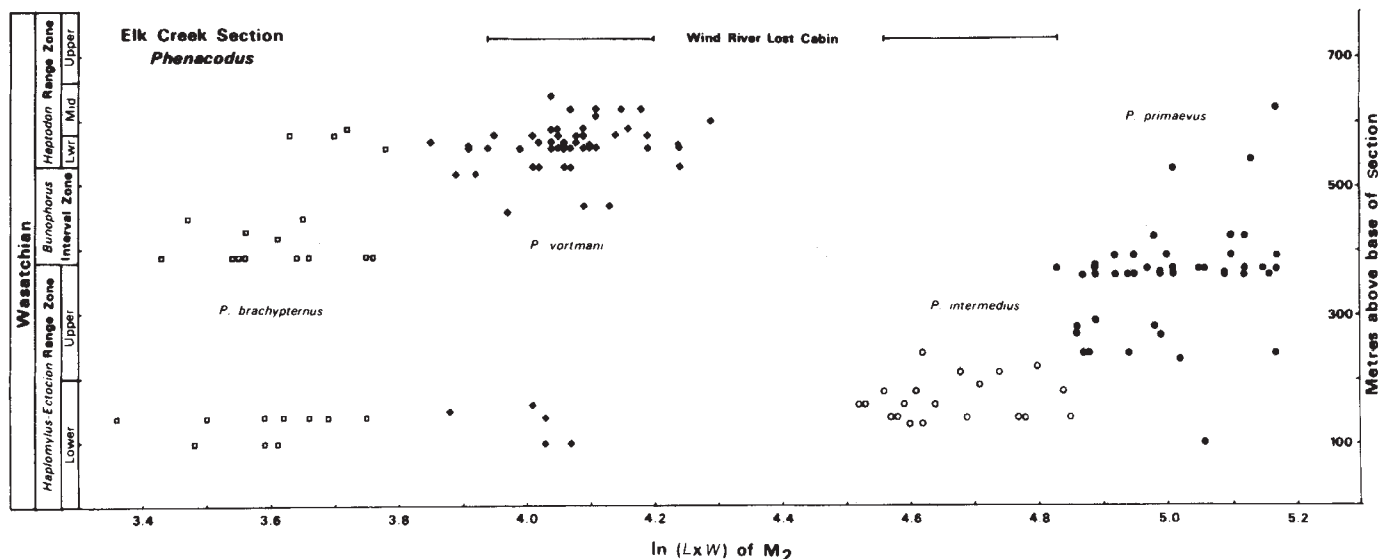


Fig. 1 Species-sequencing pattern of early Eocene *Phenacodus* in the central Bighorn Basin, Wyoming. Stratophenetic plot shows the distribution in size and time of the four species, and illustrates the discontinuity in the temporal ranges of *P. brachypternus* (□) and *P. vortmani* (◆), and the transition in dominance between *P. intermedius* (○) and *P. primaevus* (●) at the base of the Upper *Haplomyilus-Ectocion* Zone. Size is measured on the abscissa as ln of the crown area of the lower second molar (M<sub>2</sub>) and time is shown on the ordinate as metres above the base of the Elk Creek Section. The horizontal lines at the 730-m level are the maximum ranges of the two species of *Phenacodus* from the Lostcabinian of the Wind River Basin. Note the similarity between the size range of the larger species from the Wind River Basin and *P. intermedius* from the Elk Creek Section.

sample of large phenacodontid. The size of this species is similar to that of Granger's<sup>10</sup> plesiotype of *P. primaevus* and this sample is referred to as that species, as is the 100-m level outlier already mentioned. *P. primaevus* could not be derived by gradual size transformation from *P. intermedius* because both species occur together at YPM Locality 351 (evidence provided by the large variation at the 240-m level) and *P. primaevus* occurs earlier at the 100-m level. This early occurrence of *P. primaevus* also tends to rule out a speciation event as an explanation for the size

discontinuity at the 240-m level. It seems more likely that the observed pattern is the result of a shift in dominance between the two species, with the zone of transition in the interval 200–240 m. Above the 420-m level all large phenacodontids become exceedingly rare in the central Bighorn Basin. Although no  $M_2$  values substantiate this point in Fig. 1, other elements indicate that *P. intermedius* returns as the primary species of large phenacodontid in the highest part of the section. This also seems to be the case in the Wind River Basin<sup>15</sup> (Fig. 1).

**Table 1** Distribution of abundances of the five species of phenacodontid condylarths through the Elk Creek Section

| Metre level                           | <i>P. intermedius</i> | <i>P. primaevus</i> | <i>P. vortmani</i> | <i>P. brachypternus</i> | <i>E. osbornianus</i> | Sample size |
|---------------------------------------|-----------------------|---------------------|--------------------|-------------------------|-----------------------|-------------|
| 720                                   | —                     | —                   | —                  | —                       | —                     | 4           |
| 710                                   | —                     | —                   | —                  | —                       | —                     | 1           |
| 700                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 690                                   | —                     | —                   | 2                  | —                       | —                     | 28          |
| 680                                   | 1                     | —                   | 1                  | —                       | —                     | 112         |
| 670                                   | —                     | —                   | —                  | —                       | —                     | 65          |
| 660                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 650                                   | —                     | —                   | 1                  | —                       | —                     | 53          |
| 640                                   | —                     | —                   | 1                  | ?                       | —                     | 80          |
| 630                                   | —                     | —                   | —                  | —                       | —                     | 82          |
| 620                                   | —                     | 2                   | 8                  | 1?                      | —                     | 546         |
| 610                                   | —                     | —                   | 3                  | ?                       | —                     | 387         |
| 600                                   | —                     | 1                   | 3                  | —                       | —                     | 109         |
| 590                                   | 1?                    | 1                   | 5                  | ?                       | —                     | 154         |
| 580                                   | —                     | —                   | 13                 | 6                       | —                     | 359         |
| 570                                   | —                     | —                   | 8                  | ?                       | —                     | 260         |
| 560                                   | —                     | —                   | 19                 | 1                       | —                     | 395         |
| 550                                   | —                     | —                   | 1                  | —                       | —                     | 17          |
| 540                                   | —                     | 1                   | —                  | —                       | —                     | 17          |
| 530                                   | —                     | 1                   | 9                  | —                       | —                     | 424         |
| 520                                   | —                     | —                   | 1                  | —                       | —                     | 8           |
| 510                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 500                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 490                                   | —                     | —                   | —                  | —                       | —                     | 21          |
| 480                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 470                                   | —                     | —                   | 2                  | —                       | —                     | 62          |
| 460                                   | —                     | —                   | 2                  | 2                       | —                     | 83          |
| 450                                   | —                     | 1                   | —                  | 5                       | —                     | 69          |
| 440                                   | —                     | —                   | 1                  | —                       | —                     | 38          |
| 430                                   | —                     | —                   | —                  | 1                       | —                     | 16          |
| 420                                   | —                     | 2                   | 1                  | 2                       | —                     | 68          |
| 410                                   | —                     | 3                   | —                  | 1                       | —                     | 52          |
| 400                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 390                                   | —                     | 3                   | —                  | 7                       | —                     | 98          |
| <b>Upper Haplomylus-Ectocion Zone</b> |                       |                     |                    |                         |                       |             |
| 380                                   | —                     | 1                   | —                  | —                       | —                     | 5           |
| 370                                   | —                     | 9                   | —                  | —                       | 1                     | 195         |
| 360                                   | —                     | 11                  | —                  | —                       | 3                     | 209         |
| 350                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 340                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 330                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 320                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 310                                   | —                     | 1                   | —                  | —                       | 1                     | 45          |
| 300                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 290                                   | —                     | 6                   | —                  | —                       | 1                     | 153         |
| 280                                   | —                     | 2                   | —                  | —                       | 2                     | 80          |
| 270                                   | —                     | 1                   | —                  | —                       | 4                     | 111         |
| 260                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 250                                   | —                     | —                   | —                  | —                       | —                     | 7           |
| 240                                   | 1                     | 5                   | —                  | —                       | 5                     | 190         |
| 230                                   | 1                     | 1                   | —                  | —                       | —                     | 13          |
| 220                                   | 2                     | 1                   | —                  | —                       | 2                     | 69          |
| 210                                   | 1                     | —                   | —                  | —                       | 2                     | 29          |
| 200                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 190                                   | 4                     | —                   | 2                  | 1                       | 6                     | 209         |
| 180                                   | 7                     | —                   | 1                  | —                       | 2                     | 96          |
| 170                                   | —                     | —                   | —                  | —                       | —                     | 25          |
| 160                                   | 8                     | —                   | 1                  | —                       | 1                     | 109         |
| 150                                   | 2                     | —                   | —                  | 1                       | —                     | 22          |
| 140                                   | 11                    | —                   | 6                  | 8                       | 8                     | 464         |
| 130                                   | 2                     | —                   | 1                  | —                       | —                     | 26          |
| 120                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 110                                   | —                     | —                   | —                  | —                       | —                     | —           |
| 100                                   | 2                     | 1                   | 1                  | 3                       | 4                     | 157         |
| 90                                    | 1                     | —                   | 1                  | —                       | 5                     | 74          |
| 80                                    | —                     | —                   | —                  | —                       | 1                     | 14          |
| 70                                    | —                     | —                   | —                  | —                       | —                     | —           |
| 60                                    | —                     | —                   | —                  | —                       | —                     | —           |
| 50                                    | —                     | —                   | —                  | —                       | —                     | 20          |
| 40                                    | —                     | —                   | —                  | —                       | —                     | —           |

The abundances of the five species and the total sample size at each level are given as minimum number of individuals. Note the absence of *P. brachypternus* and *P. vortmani* from the entire Upper Haplomylus-Ectocion Zone (200–380 m).



This temporal discontinuity in the range of *P. intermedius* is even more evident in the ranges of *P. brachypternus* and *P. vortmani*. *P. brachypternus* is distinguished from all other species of *Phenacodus* by its small size and elongate lower fourth premolar ( $P_4$ ) (Fig. 2). This condition of  $P_4$  is present in the samples from all levels. As indicated in Fig. 1, there are no specimens of *P. brachypternus* from the entire 200–380-m interval (Upper *Haplomylus*–*Ectocion* Zone). The relative abundance of *P. brachypternus* through the entire section is 0.006 (Table 1). Using this as the most conservative estimate the probability of *P. brachypternus* not being found in the Upper *Haplomylus*–*Ectocion* Zone, if it were in fact present, is 0.0007. Of course, the higher frequency of *P. brachypternus* in the two zones bounding the zone of absence gives a lower probability that the species was in fact present in the intervening zone and has not yet been found. It is possible that the species present in the Lower *Haplomylus*–*Ectocion* Zone became extinct and a totally new species appeared at the beginning of the *Bunophorous* interval zone; this is highly unlikely in this particular case because of the presence of the unique  $P_4$  in both the lower and upper samples, as noted above. The pattern for *P. vortmani* is very similar to that of *P. brachypternus* and an equally convincing argument can be made for its absence from the Upper *Haplomylus*–*Ectocion* Zone. However, for *P. vortmani* there is no unifying morphology between the lower and upper samples, and the positing of the discontinuity in the temporal range of this species is based on inference from *P. brachypternus*.

The temporal gap in the range of *P. brachypternus* is on the order of 200 m (slightly more for *P. vortmani*) and probably represents a time interval of ~1 Myr. In searching for an explanation for the species-sequencing patterns of these two species, it is necessary to consider the changes occurring in the entire fauna as well as aspects of the history of the Phenacodontidae. Phenacodontids experienced a severe decrease in relative abundance across the Clarkforkian–Wasatchian boundary<sup>16</sup>. This decline continued through the Wasatchian and correlates with the appearance and rise of the more advanced Perissodactyla. During the Wasatchian, larger condylarths are characterized by a patchy distribution such that the presence of one often means the exclusion of others<sup>17</sup>. In the central Bighorn Basin, the lower and upper boundaries of the Upper *Haplomylus*–*Ectocion* Zone (Biohorizons A and B respectively) are times of increased faunal turnover<sup>7</sup>. Biohorizon B also seems to correlate with a shift in the environmental regime as indicated by changes in the depositional environments and palaeoflora across this boundary<sup>18</sup>. No significant shift in the palaeoflora has been documented across Biohorizon A, although a slight shift is observed in the depositional environment.

It is informative to consider the distribution of a fifth species of phenacodontid, *Ectocion osbornianus*. At the base of the Wasatchian, *Ectocion* is equal in size with *P. brachypternus*. It increases slightly in size through the *Haplomylus*–*Ectocion* Zone such that at the top of its range it is intermediate in size between *P. brachypternus* and *P. vortmani*. During the Lower *Haplomylus*–*Ectocion* Zone, *P. brachypternus* and *P. vortmani* decrease in abundance just before their disappearance from the central basin—this decrease is matched by an increase in abundance of *Ectocion*. The reciprocity of abundances is not continued into the Upper *Haplomylus*–*Ectocion* Zone, when *Ectocion* abundance is also reduced. *Ectocion* becomes locally extinct in the central basin at the top of the Upper *Haplomylus*–*Ectocion* Zone, just before the return of *P. brachypternus* and *P. vortmani*.

Thus it seems that during the Wasatchian, phenacodontid condylarths became relatively sensitive environmental indicators. The general increase in open habitats through the Wasatchian (L. Hickey and S. Wing, personal communication) was probably responsible for the decline in abundance of the phenacodontids and the rise of the perissodactyls. Due to their dietary preferences (frugivore–foliivores), the phenacodontids were better adapted to a closed canopy forest habitat, whereas the perissodactyls (foliivores) were favoured in more open habitats (C. Janis and P. Gingerich, personal communication).

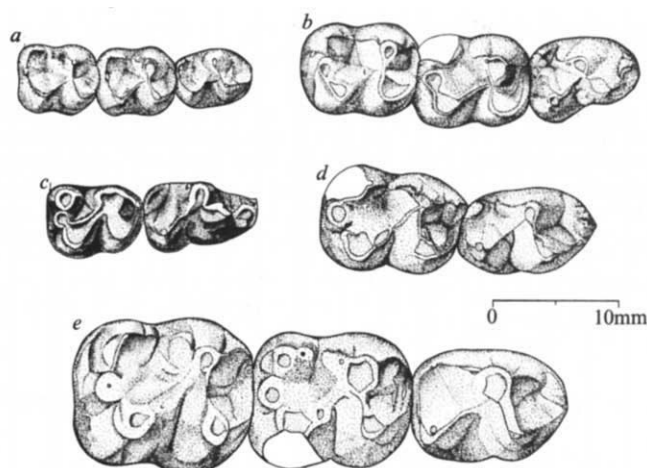


Fig. 2 Comparison of the five species of phenacodontid condylarths from the Elk Creek Section of the central Bighorn Basin. a, *Ectocion osbornianus* (YPM 21211); b, *Phenacodus vortmani* (YPM 31949); c, *P. brachypternus* (YPM 22963); d, *P. intermedius* (YPM 32416); e, *P. primaevus* (YPM 25502). All specimens show right  $P_4$ – $M_1$  or  $P_4$ – $M_2$  in occlusal view and represent only a portion of the preserved specimen. Note the similarity in size between *Ectocion* and *P. brachypternus*, and the elongated  $P_4$  of *P. brachypternus*.

The exclusion and subsequent return of the two small *Phenacodus* species may be attributed to an increase in competition with *Ectocion*. That this increase in competition was influenced by external factors is seen in the later decline of *Ectocion*, and the correlation of the species-sequencing events with high species turnover rates and changing environments at Biohorizons A and B. The shifts in the environment that led to these events were probably of a more subtle nature than those that led to the general decline of the Phenacodontidae.

Documentation of the discontinuity in the temporal range of *Phenacodus brachypternus*, with its unifying morphology of disjunct samples, is important because it allows recognition of gaps in the distribution of species where the morphology is ambiguous, as is the case for *P. vortmani*. Preliminary work indicates that coincident gaps also occur in the ranges of the condylarth *Hyopsodus* cf. *simplex*, in a species of the perissodactyl genus *Hyracotherium*, and to a lesser degree in the primate *Pelycodus mckennai* and the arctocyonid *Thryptacodon antiquus*.

More significantly, perhaps, the temporal gaps demonstrate the importance of the biogeographical or environmental component in influencing the species-sequencing patterns in a single basin. The first appearance of a lineage within a stratigraphical sequence cannot necessarily be treated as a speciation event but might be regarded rather as immigration, either from a different geographical area or a previously unsampled habitat. The phyletic linkage of a species with its nearest stratigraphical congener is not necessarily the most parsimonious of possible evolutionary patterns. Documentation of discontinuities in the morphology of successive species within a single basin offers no real evidence for either phyletic gradualism or punctuated equilibria. The long-term stasis in morphology and size shown by the four species of *Phenacodus* conforms to the pattern expected in a model of evolution by punctuated equilibria. However, shifting geographical ranges under the influence of a changing environment, rather than speciation, seems to be an adequate explanation for the observed pattern.

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## Abolition of seasonal embryonic diapause in a wallaby by pineal denervation

Marilyn B. Renfree\*, D. W. Lincoln†, O. F. X. Almeida‡ & R. V. Short‡

\* School of Environmental and Life Sciences, Murdoch University, Western Australia 6150, Australia

† Department of Anatomy, The Medical School, University of Bristol, Bristol BS8 1TD, UK

‡ MRC Reproductive Biology Unit, 37 Chalmers Street, Edinburgh EH3 9EW, UK

Embryonic diapause is a widespread phenomenon in mammals and may be controlled either by lactation or by seasonal environmental factors<sup>1</sup>. A few species, such as the tammar wallaby, *Macropus eugenii*, use both mechanisms, depending on the time of year. On Kangaroo Island, South Australia (latitude 36° S), female tammar give birth to a single young in late January or early February<sup>2</sup>. Mating occurs on the day after birth and the resulting conceptus grows to a 100-cell blastocyst before entering a state of embryonic diapause. This arrest of embryonic growth is due to a suppression of the corpus luteum by prolactin induced by the young in the pouch sucking the teat (lactational diapause)<sup>3,4</sup>. If the young is removed from the teat between February and early June, the corpus luteum hypertrophies, the blastocyst resumes growth and birth occurs 27 days later<sup>5</sup>. In the second half of the year (June to December), diapause is not solely dependent on the sucking stimulus as it is maintained after weaning, which usually occurs in October, or following experimental removal of the pouch young. This is referred to as seasonal diapause<sup>5</sup>. Reactivation occurs on or about the summer solstice (22 December) and there is evidence that it is photo-periodically induced<sup>6</sup> (Fig. 1). We report here that bilateral removal of the superior cervical ganglion in female tammar eliminates seasonal embryonic diapause, but has no effect on lactational diapause. This is the first evidence that the pineal gland may be involved in regulation of embryonic diapause in mammals.

Twelve wallabies in lactational diapause with pouch young of similar ages were operated on in March and April and the first week in May 1980. In eight of these animals, the superior cervical ganglia were removed bilaterally, fixed in glutaraldehyde and examined histologically to confirm the presence of ganglion cells. Four animals served as sham-operated controls and a further nine unoperated animals were maintained in parallel. The animals were kept in a large outdoor enclosure together with three males at Murdoch University, Western Australia (latitude 32° S). To examine the effects of ganglionectomy on pineal function, blood samples were collected from a lateral tail vein at 12.00 and 24.00 h on the day before surgery and again some weeks after the operation, and the levels of melatonin determined by a modified radioimmunoassay<sup>7</sup>.

**Table 1** Effect on embryonic diapause of the bilateral removal of the superior cervical ganglia from the tammar wallaby

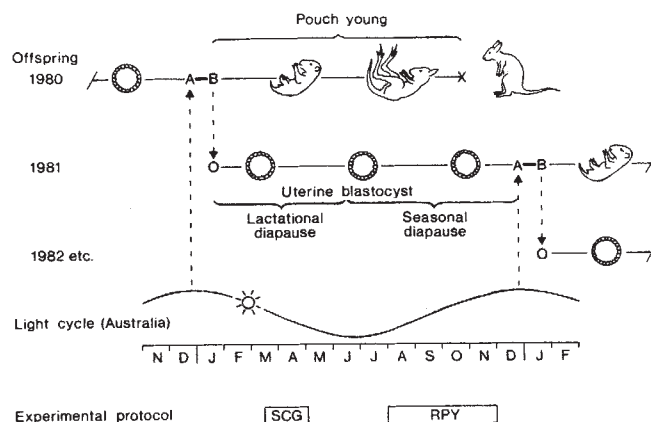
| Treatment group                     | No. of mothers with small pouch young in February 1980 | No. of young born May to July 1980, pouch young of February left <i>in situ</i> | No. of young born August to November 1980 | Mothers giving birth to new young in February 1981 (Young of November left <i>in situ</i> ) |
|-------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------|---------------------------------------------------------------------------------------------|
| Ganglionectomy (March to early May) | 8                                                      | 0                                                                               | 24                                        |                                                                                             |
| Sham-operated* (March to early May) | 4                                                      | 0                                                                               | 0                                         | 9/12                                                                                        |
| Unoperated                          | 9                                                      |                                                                                 |                                           |                                                                                             |

\* Number reduced to three in September when one wallaby was culled due to an eye infection.

Further plasma samples were taken every 3 h over a 27-h period in August and November.

The removal of the superior cervical ganglia had no effect on lactational diapause. In late July, four of the ganglionectomized wallabies lost their pouch young, two of which were recovered dead; these two were small for their age. This was also the case for all the pouch young still carried by the other ganglionectomized animals. All eight ganglionectomized animals had smaller mammary glands than did control animals, suggesting that the operation had interfered with mammary development.

To determine whether ganglionectomy had affected seasonal diapause, pouch young were removed from the wallabies on 1 August (Table 1). This resulted in the immediate resumption of blastocyst growth in seven of the eight ganglionectomized wallabies, and they gave birth 27-28 days later. They subsequently came into oestrus, ovulated and their blastocysts entered lactational quiescence. By contrast, none of the 13 controls gave birth. During the next 4 months of seasonal diapause, any new pouch young were removed every 30-35

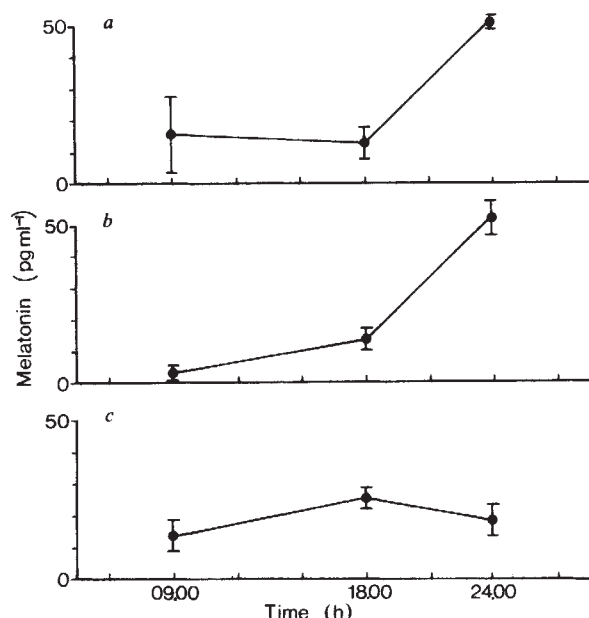


**Fig. 1** Diagrammatic illustration of the reproductive pattern of the tammar wallaby *M. eugenii*, and the experimental protocol used to study the effect of removing the superior cervical ganglia on embryonic diapause. Ovulation, mating and fertilization (O) occur within 24 h of giving birth (B) but the blastocyst so formed normally remains in a state of diapause (suspended animation) for 11 months induced initially by the sucking of the pouch young ('lactational diapause'). Later in the year diapause is maintained in the absence of a pouch young by seasonal constraints ('seasonal diapause'). At about the time of the summer solstice, the blastocyst recommences growth (A) and a baby weighing <0.5 g is born 27 days later (B). The object of the experiment was to determine whether removal of the superior cervical ganglia (SCG) from the mother would influence the duration of seasonal diapause after the removal of the sucking pouch young (RPY).



days. All eight ganglionectomized wallabies continued to give birth and ovulate, producing 24 newborn young, whereas the controls produced none. One of the sham-operated animals had to be killed in September because of an infected eye: this animal carried a diapause blastocyst in the uterus. Nine of the remaining twelve control animals gave birth at the normal time in February 1981, proving that their blastocysts had also remained in diapause after removal of pouch young in August.

A marked diurnal rhythm was observed in the plasma level of melatonin in intact wallabies, with the midnight values being ~three- to fivefold higher than those around midday. These animals apparently showed a seasonal change: the midnight values were  $37.8 \pm 9.5$  pg ml<sup>-1</sup> ( $\pm$ s.e.m.) in May,  $138.1 \pm 29.7$  in August and  $52.0 \pm 4.0$  in November. The bilateral removal of the superior cervical ganglia clearly altered pineal function as it completely abolished the nocturnal rise in melatonin, although melatonin was still present in the plasma at a mean level of  $19.2 \pm 2.6$  pg ml<sup>-1</sup> (Fig. 2).



**Fig. 2** Plasma melatonin concentrations (mean  $\pm$  s.e.m.) in sham-operated (a), intact (b) and ganglionectomized (c) female tammar wallabies bled at 09.00, 18.00 and 24.00 h in November. Ganglionectomy abolishes the night-time increase in melatonin concentration.

Although the tammar wallaby is a marked seasonal breeder, it is unusual that the male shows no seasonal changes in testicular size or fertility, and the female is capable of ovulating throughout the year provided there is no corpus luteum in the ovary<sup>3,4</sup>. The maintenance of a relatively inactive corpus luteum during the 11 months of embryonic diapause is not due to a deficiency of luteinizing hormone or follicle-stimulating hormone because hypophysectomy reactivates the corpus luteum and causes a resumption of embryonic development at all times of the year<sup>8</sup>. However, diapause can be maintained in the hypophysectomized animal by prolactin injections; thus it seems that this is the key pituitary hormone in diapause control<sup>3,4</sup>. Furthermore, prolactin concentrations are elevated during seasonal diapause<sup>9</sup>. Melatonin is known to stimulate prolactin secretion in some eutherian mammals<sup>10</sup>, while pinealectomy or removal of the superior cervical ganglia disturbs the normal seasonal changes in prolactin secretion<sup>11,12</sup>. Our observation that ganglionectomized wallabies had small mammary glands and emaciated pouch young when examined in late July suggests that ganglionectomy also interferes with the seasonal increase in prolactin secretion in the wallaby.

Thus our results suggest that in the tammar wallaby seasonal embryonic diapause, but not lactational diapause, depends on the pineal gland. Abolition of the diurnal rhythm of melatonin secretion by superior cervical ganglionectomy may suppress the

seasonal rise in prolactin secretion, thereby allowing reactivation of the corpus luteum and a resumption of embryonic growth.

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## Moving and the motion after-effect

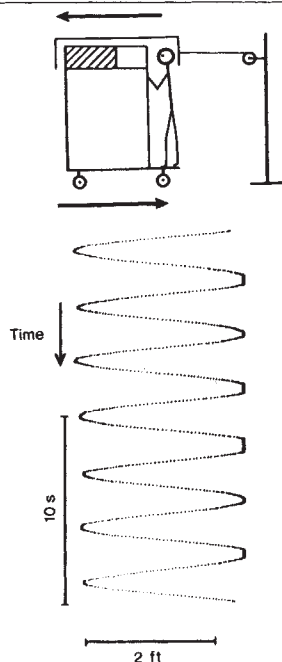
L. R. Harris, M. J. Morgan & A. W. Still

Department of Psychology, University of Durham,  
Science Laboratories, South Road, Durham DH1 3LE, UK

The movement after-effect (MAE) is caused by inspecting a pattern in which many stimulus elements in the visual field are in coherent movement; after inspection, stationary elements seem to move in the opposite direction. By far the commonest cause of such a retinal stimulus is movement of the observer, not movement of the environment. We suggest here, therefore, that the usual laboratory stimulus for inducing the MAE presents the observer with conflicting sensory cues. The optical input is normally associated with self motion, but other cues such as the vestibular input simultaneously tell the observer that he is stationary. In these circumstances a recalibration of the relationship between optical and other information might occur and we suggest that the after-effect may be at least in part a consequence of this recalibration, rather than being entirely due to a passive fatigue-like process.

The hypothesis that the MAE is due to recalibration clearly predicts that if inspection of the moving pattern occurs in circumstances where there is not an incompatibility between visual and other information, then the MAE will be much reduced. In other words, there will be reduced adaptation if the visual movement is appropriately correlated with the observer's movements. Evidence for this is the surprising lack of a marked MAE after driving a car. To test our prediction we used the expanding/contracting MAE, in which the adapting stimulus consists of elements in centripetal or centrifugal motion respectively. Inspection of centrifugal motion results in a centripetal after-effect, and vice versa<sup>1</sup>. Of course, centrifugal motion of the visual world is normally produced by forward locomotion; thus we should expect from our hypothesis that adaptation would be reduced if this pattern of visual movement was indeed produced by forward bodily movement. Similarly, adaptation to centripetal visual movement should be reduced if the visual flow was produced by backward bodily movement. The other two combinations, centripetal+forward movement and centrifugal+backward motion, should, if anything, increase the after-effect because they provide even stronger incompatibilities than the normal case.

In our experiment, both the observer and the display were mounted on a trolley that was moved backwards and forwards by one of the experimenters. The screen was thus always 0.3 m away from the observer and always subtended  $16.7 \times 22.7$  deg. This is shown diagrammatically at the top of Fig. 1. By monitoring the movement of the trolley and using a computer to



**Fig. 1** The subject stood on a trolley and viewed an oscilloscope (hatched box) that was also mounted on the trolley. The trolley was attached by a string to a stationary potentiometer which passed to the computer a voltage corresponding to the subject's position. The observer and screen were under a dense black shroud. The figure also shows a typical computer record of a subject being pushed backwards and forwards approximately sinusoidally. We were able to push smoothly at  $\sim 0.4$  Hz throughout the 10-min adaptation period.

generate expansion in the display based on this movement, we were able to control precisely the compatibility between the visual cue to motion and the observer's real motions (see Fig. 1). The observer and oscilloscope were covered by a shroud and the screen was viewed monocularly through a 1.5 log unit neutral density filter. In these conditions the edge of the oscilloscope was only dimly visible and then only when the display was on. The display was viewed monocularly to reduce cues as to the real distance of the screen.

The display consisted of an array of 400 randomly positioned dots on a Hewlett-Packard 1333A display oscilloscope. To produce an expanding stimulus the dots were initially confined to a  $2.2^\circ$  square, which was then continuously expanded to a maximum of  $11.0^\circ$  by multiplying the  $x$  and  $y$  coordinates of each dot by a voltage proportional to the observer's position, using the digital-to-analog converters of the Alpha minicomputer which controlled the display. The program was arranged such that the maximum size was reached at the furthest forward extent of the observer's movement. Contracting motion was produced by the reverse of this process.

The subject was pushed repetitively forwards and backwards along a short run  $\sim 1$  m long. The expansion of the pattern on the screen simulated the retinal expansion that would have occurred had the subject been pushed towards a stationary display at the far end of the room. The display was blanked except for a fixation dot while the trolley was moving backwards. An adaptation trial lasted 10 min and typically included  $\sim 200$  movement cycles. The magnitude of the MAE was then measured by the method of cancellation (see below). On the following day, the magnitude of the MAE was again determined, after exactly the same display expansion as before but with the trolley stationary.

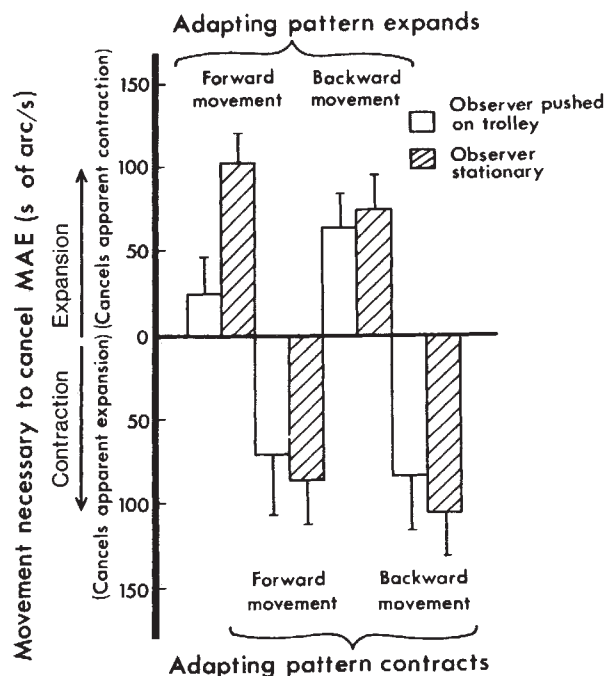
In addition, the experiment was repeated with a contracting pattern visible only during the backward phase of the trolley movement cycle. The control for this was the presentation of an identical contracting pattern when the observer was stationary. Finally, we looked at the after-effects produced by the two incompatible combinations: forward motion with a contracting display and backward motion with an expanding display. These were arranged by programming the computer to scale the

pattern as if the observer were moving in the reverse of the true direction. In all these conditions the pattern was blanked while the trolley was being moved to its starting position.

The size of the resulting MAEs was measured by the method of cancellation. Before and after each adaptation session the computer presented a  $2.2^\circ$  square display of 400 random dots which expanded or contracted depending on the setting of a potentiometer under the observer's control. The display was repeatedly presented for 1.4 s with intervals of 1.4 s of a blank screen. The brief exposure made it less likely that displacement was being detected rather than movement. The observer's task was to set the potentiometer so that the pattern appeared neither to expand nor to contract. He had five exposures in which to make a setting, at the end of which the objective expansion/contraction in the pattern was taken as the measure of the after-effect. This procedure was repeated 10 times and the results averaged. A random offset was added to the potentiometer on each of the 10 trials to avoid learning effects. The initial presentation on each trial alternated between expansion and contraction.

The experiment was carried out using five normally sighted observers, including ourselves, each of whom used their preferred eye for viewing the display monocularly. All the conditions and their appropriate controls (observer stationary) were repeated at least twice by each observer and the mean results are shown in Fig. 2. The main finding was the very small MAE obtained after viewing an expanding pattern during forward movement in contrast to the larger after-effects found in the other conditions.

These results were not entirely predicted, as they failed to show both the expected enhancement of the MAE by incompatible conditions of self motion and a marked suppression of the after-effect of a contracting pattern seen during backward self movement. They do, however, reveal that self motion can have a pronounced effect on what has been considered previously as an entirely visual phenomenon. Our explanation of the data is that normal experience with forward locomotion leads to a very fine



**Fig. 2** The amplitude of the after-effects obtained in each of the eight forwards/backwards, expanding/contracting, moving/stationary combinations. These are the averages ( $\pm$  s.d.) of at least two repetitions of all eight conditions for five subjects with 10 settings at the end of each adaptation period. Trials were only carried out if the subject was initially able to set the zero point to  $< 14$  arc s per s of contraction or expansion. Analysis of variance showed a significant interaction ( $F_{1,4} = 24.83$ ,  $P < 0.001$ ) between the effect of self motion and its direction.



calibration of the relationships between the expanding visual field and other cues to forward motion. As long as the correct metrical relationship between the two is maintained, there will be no MAE. The absence of any marked suppression of the MAE produced by visual contraction during backward motion may be due to lack of normal experience of this condition.

These experiments with varying correspondence between actual movement and visual movement show that active recalibration of the visual and vestibular systems is an integral part of the mechanism underlying the classical movement after-effect.

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## Genesis of rods in teleost fish retina

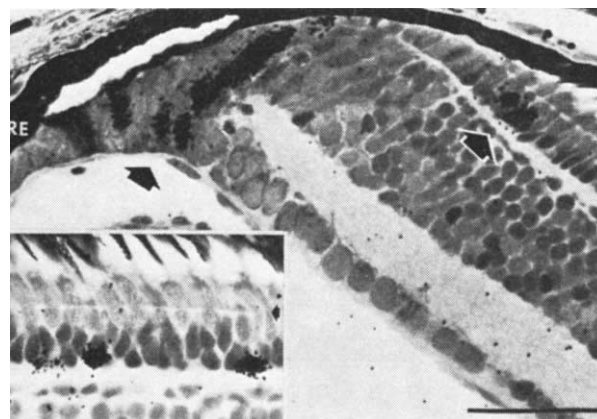
Pamela Raymond Johns & Russell D. Fernald

Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA

Department of Biology, University of Oregon, Eugene, Oregon 97403, USA

**Fish grow throughout life<sup>1</sup> and new neurones are added to the retina as the eyes increase in size<sup>2-6</sup>. As the retina expands, the density of cells decreases: ganglion cells, cones and cells in the inner nuclear layer are spaced further apart in retinas from larger (older) fish<sup>2-5,7-9</sup>. In contrast, the density of rods increases during larval development and is then maintained approximately constant as the adult eye grows<sup>2-5</sup>. Previous developmental studies, in which <sup>3</sup>H-thymidine was used to identify proliferating cells, revealed a germinal zone at the margin of the retina. The germinal cells divide to produce new retinal neurones which are added annularly at the perimeter of the growing retina<sup>5,6,10</sup>. A similar circumferential pattern of growth has been demonstrated in larval amphibians<sup>11-15</sup>. These studies concluded that the retinal margin is the only site of neurogenesis in post-embryonic retinas. In contrast, our observations suggest that new rods originate from mitotic divisions of precursor cells which are interspersed among the nuclei of mature rods within the retina. The selective addition of rods throughout the retina could explain how the proportion of rods relative to other neurones increases as the retinas of fish grow<sup>2-4,7</sup>. Preliminary reports of these experiments have appeared elsewhere<sup>16,17</sup>.**

Juvenile and adult specimens of an African cichlid teleost fish (*Haplochromis burtoni*) and of common goldfish (*Carassius auratus*) were injected intraperitoneally with one or several doses of <sup>3</sup>H-thymidine. This metabolic precursor of DNA is incorporated exclusively into the nuclei of proliferating cells<sup>18</sup>. Thus, in autoradiographs at short survival times the nuclei of dividing cells are labelled, and at longer survival times the label occurs in nuclei of differentiated cells, the progeny of those labelled earlier<sup>19</sup>. In retinas examined autoradiographically after survival times of 1 day or less, label was observed in nuclei of dividing cells clustered in the circumferential germinal zone (Fig. 1). We expected this from previous descriptions of <sup>3</sup>H-thymidine labelling in retinas of fish<sup>5,6,10</sup>. In addition, however, we saw scattered labelled nuclei more centrally, within regions of the retina that had already differentiated. Some of these could be identified as glia (in the optic fibre and ganglion cell layers) and others were endothelial cells or other vascular elements. A few labelled nuclei in the inner nuclear layer could not be identified with certainty in these preparations. Labelled, non-neuronal nuclei were more prevalent in the retinal preparations from goldfish than in those from *Haplochromis*; we cannot explain this difference between the two species of fish. In both goldfish and *Haplochromis*, most of the labelled nuclei that were not in the circumferential growth zone were in the outer nuclear layer, a neural layer which contains neither glia nor vascular nuclei, but only the nuclei of photoreceptor cells (Fig. 1, inset).

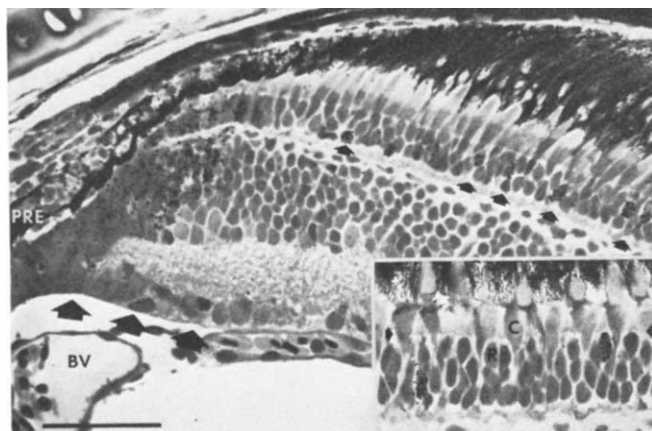


**Fig. 1** An autoradiograph of the retinal margin in a juvenile goldfish injected with <sup>3</sup>H-thymidine. This fish, which weighed 1.7 g, was injected intraperitoneally with 20  $\mu$ Ci per g <sup>3</sup>H-thymidine (104 Ci mmol<sup>-1</sup>). Four hours later it was anaesthetized and killed by decapitation. The eyes were enucleated and fixed in buffered aldehydes. The tissue was embedded in methacrylate and meridional sections of the retina were prepared for autoradiography. Several nuclei in the germinal zone at the margin of the retina had incorporated the label and are covered by black silver grains (left arrow). In addition, there are two labelled nuclei in the outer nuclear layer of the retina (right arrow). The pigmented retinal epithelium (PRE) surrounds the neural retina. Scale bar, 50  $\mu$ m. Inset: three labelled nuclei in the outer nuclear layer of the same retina are shown at higher magnification. They all lie along one edge of the outer nuclear layer, at the border of the outer plexiform layer. Labelled nuclei in the youngest (peripheral) retinal regions were often located along this edge, but those in more central regions were found at all levels within the outer nuclear layer. Small arrows indicate external limiting membrane.

Many of the labelled nuclei in the outer nuclear layer were in peripheral regions of the retina, but in all preparations, at least a few labelled nuclei were in the central retina as well. Cells with an ultrastructural appearance similar to that of the germinal cells at the margin have been described by one of us<sup>16</sup> in electron micrographs of the outer nuclear layer of the peripheral retina in young goldfish. It is likely that these undifferentiated cells are the ones that were labelled by <sup>3</sup>H-thymidine at short survival times.

Other retinas from both *Haplochromis* and goldfish were examined at longer survival times (weeks or months later). The progeny of the proliferating cells in the circumferential germinal zone had by then differentiated into neurones (Fig. 2). The labelled neurones produced at the margin remained clustered in an annulus, consistent with previous reports<sup>5,6,10-15</sup>. As for the scattered labelled nuclei within the retina, those in the outer nuclear layer increased in number and persisted for several months, the longest survival time that we examined. These cells differentiated into rods as judged by the following cytological criteria: their nuclei were elongated, often spindle-shaped, dense and homogeneously stained, surrounded by little visible cytoplasm and located in the outer nuclear layer vitread to the external limiting membrane (Fig. 2, inset). These characteristics match previous descriptions of rods in teleost fish<sup>20,21</sup>. Nuclei of rods were the only labelled neurones not confined to the circumferential growth zone.

In general, dividing cells in the outer nuclear layer were easier to demonstrate in retinas from younger and rapidly growing fish. The cichlid, *Haplochromis*, was particularly useful for this study, because even adults could be induced to grow rapidly by appropriate manipulation of environmental and social conditions<sup>22</sup>. Nevertheless, the numbers of labelled nuclei we observed in the outer nuclear layer varied greatly among individual fish at equivalent survival times, even when identical amounts of <sup>3</sup>H-thymidine were administered in multiple doses in an attempt to minimize variability due to leakage or a misplaced needle. Individual growth rates vary tremendously in fish<sup>1</sup>, and if the eye does not grow, the number of retinal cells does not increase<sup>2,5</sup>. It is possible that the rate of neuronal addition is controlled at the level of mitotic activity in the retinal germinal cells, and thus the



**Fig. 2** An autoradiograph of the retina from a juvenile goldfish injected 20 days previously with 30  $\mu\text{Ci}$  per g  $^3\text{H}$ -thymidine (95 Ci  $\text{mmol}^{-1}$ ), administered in a series of six injections at 8-h intervals. A narrow strip of retina adjacent to the margin contains labelled nuclei (large arrows) in all retinal layers. Only in the outer nuclear layer do labelled nuclei spread towards central retina (small arrows). PRE, pigmented retinal epithelium; BV, blood vessel. Scale bar, 50  $\mu\text{m}$ . Inset: two labelled nuclei in the outer nuclear layer are shown at higher magnification. They are cytologically indistinguishable from the surrounding nuclei of rods (R). The nuclei of cones (C), in contrast, are larger, paler and lie in a row at the level of the external limiting membrane (arrows). Nuclei of cones were never labelled, except in the circumferential growth zone.

numbers of nuclei labelled by  $^3\text{H}$ -thymidine in each retina may be related to the rate of growth of that fish.

We have demonstrated that new rods originate from mitotic divisions of precursors whose nuclei reside in the outer nuclear layer. This insertion of new rods within differentiated retina is consistent with the previous observation that the density of these cells remains constant as the adult retina expands<sup>3-5</sup>. However, the present experiments did not indicate whether the numbers of new rods added were sufficient to counteract exactly the spreading of cells which accompanies retinal growth. Various other hypotheses have previously been proposed to explain the increment in number of rods during growth of the teleost retina. All require movement of cells, either from one retinal layer into another, or from the marginal germinal zone into central regions. For example, Lyall<sup>3</sup> and Blaxter and colleagues<sup>23-25</sup> suggested that cells from the inner nuclear layer might be recruited by the outer nuclear layer and once there differentiate into rods. Another idea, first proposed by Bernard<sup>26</sup> in a study of retinal development in amphibians, and later revived by Lyall<sup>27</sup> to explain the accumulation of rods in retinas of larval trout, is that cones might be transformed into rods during development. In contrast to these other hypotheses, we suggest, very simply, that new rods are born where they are needed, within the outer nuclear layer throughout the retina.

Previous autoradiographical studies of retinal growth have shown dispersed labelled nuclei not confined to the circumferential growth zone, especially in the outer nuclear layer, in goldfish (refs 5, 6 and C. Stürmer, personal communication) and in another cyprinid teleost related to goldfish<sup>10</sup>. Scholes<sup>10</sup> suggested that these labelled nuclei were rods produced by mitotic divisions within the outer nuclear layer adjacent to the margin, whereas Johns<sup>5</sup> concluded that new rods originated in the germinal zone at the margin but were subsequently displaced towards the central retina, in accord with Müller's<sup>2</sup> hypothesis. The present results are more consistent with Scholes' interpretation, because many of the labelled nuclei that we saw in the outer nuclear layer were too far from the margin to have moved from the circumferential germinal zone within the allotted survival time of 24 h or less.

We have not yet investigated the intriguing problem of why only rods are added to already differentiated regions and why they are kept at constant density as the retina grows. We speculate that this may be necessary to maintain constant visual

sensitivity, as the density of photopigment would thereby be preserved in all sizes of retinas. The cones, unlike the rods, do spread apart during growth, but this probably does not affect their function. Visual acuity is dependent on both the spacing of cones and the size of the retinal image<sup>28</sup>, and the size of the image enlarges proportionally as the eye grows<sup>29</sup>. Thus, even though the cones spread apart, the eye maintains an equivalent resolving power. We find no evidence that new cones are inserted into the growing retina in the way that rods are.

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**Note added in proof:** Sandy and Blaxter<sup>30</sup> have recently reported that in retinas of larval herring and sole, rods are generated by dividing 'basal' cells located in the outer nuclear layer. One of us (P.R.J.) has found similar cells in retinas of larval goldfish<sup>31</sup>. It would therefore appear that the genesis of rods in the adult teleost retina follows a pattern established during larval development.

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## Visual localization after rearrangement of retinotectal maps in fish

D. P. M. Northmore

Institute for Neuroscience, University of Delaware, Newark, Delaware 19711, USA

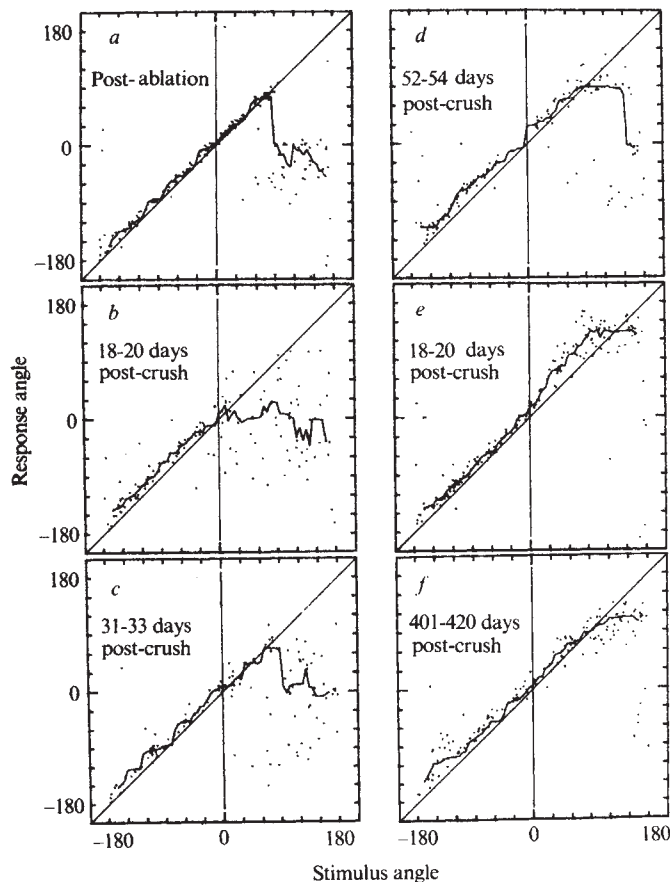
In each lobe of the optic tectum, the primary visual centre of fishes, terminals of the optic nerve distribute themselves to form a precise visuotopic map of the visual field seen by the opposite eye<sup>1,2</sup>. The tectum has an essential role in orienting a fish towards a food object before the fish seizes it<sup>3,4</sup>. Moreover, experiments involving selective lesions or focal stimulation of tectum suggest that the detection and localization of an object in space depend on the visuotopic map, and that the fish's orienting response is directed by a 'motor map' in register with the visuotopic map<sup>5-9</sup>. The relationship between the two maps has been studied here by taking advantage of the finding in goldfish that the removal of the caudal half of the tectum leads in time to the formation of a compressed but functional visuotopic map<sup>7,10,11</sup>. As behavioural orientation was found to be accurate despite compression, the putative motor map cannot be fixed anatomically in relation to the tectum.



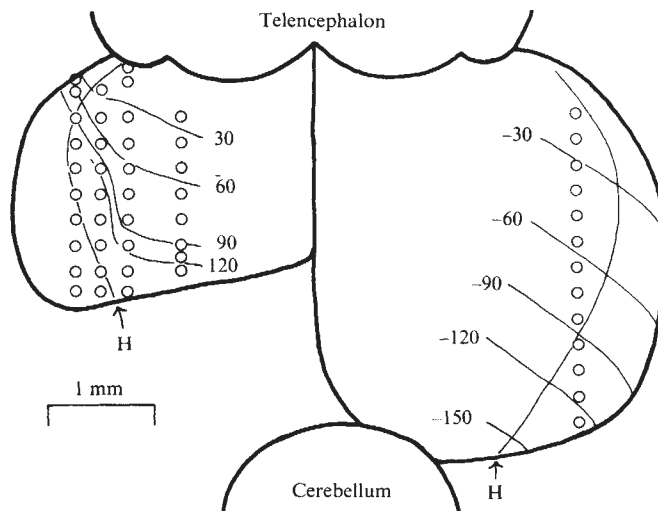
To study the visual field defects and disturbances of orientation to visual stimuli caused by surgery, a behavioural perimetry technique was developed for fish<sup>8</sup>. They were first trained with food rewards to swim to a patch of light (1 cm diameter) projected at one of eight positions on the walls of a circular tank (64 cm diameter). As the aim was to obtain ballistic orientation to the apparent stimulus position, the stimulus was flashed (125 ms duration) so as to be invisible by the time the fish started turning towards it. On each trial, a stimulus and a response angle were calculated from television pictures of the fish loaded every 133 ms into the memory of a computer. The stimulus angle was the bearing of the stimulus flash, and the response angle was the bearing of the place on the wall of the tank to which the fish was oriented 0.8 s after the flash, both angles being computed with respect to the fish's body axis and head position at the time of the stimulus flash.

Of several species of fish tested, members of the sunfish family oriented most accurately and consistently<sup>8</sup>. Although the results described here were obtained from one 11-cm pumpkin seed sunfish (*Lepomis gibbosus*), they were qualitatively similar to those obtained from two other sunfish that were tested for nearly a year after surgery.

After pretraining in the perimeter, the caudal half of the left tectum was ablated as a first step towards inducing compression of the retinotectal map. The behavioural data collected over the succeeding 13 days (Fig. 1a) revealed a failure to orient or



**Fig. 1** Orientation data from visuomotor perimetry performed on a sunfish at different stages during the reorganization of its retinotectal connections. The graphs show the response angle (deg) 0.8 s after a stimulus flash as a function of the stimulus angle, each point representing the outcome of a single trial. The curves drawn through the points are running medians of response angle computed from a 30°-wide boxcar moved along the stimulus angle axis. The diagonal lines represent ideal responses (that is, stimulus angle = response angle) and the vertical lines separate left from right visual fields. *a* Shows all data collected after ablation of the caudal half of the left tectum, but before crushing the right optic nerve 13 days later. *b-f* Show orientation data collected at successive times after optic nerve crush.



**Fig. 2** Results of mapping the visuotopic projection on the optic tectum of the sunfish whose behavioural data are shown in Fig. 1. The normal right tectum and the half-ablated left tectum are shown in plan view lying between the telencephalon and cerebellum. Electrophysiological mapping was done with eyes in air 421 days after crushing the right optic nerve. A microelectrode recorded multi-unit activity in the superficial layers of the tectum at the circled positions. The region of the visual field in which a hand-held black spot evoked neural activity at each electrode position was marked on a transparent hemisphere centred on the contralateral eye. From these field positions, the projection of certain meridia of visual space were plotted on the tectal surface. The lines labelled H are the projections of the horizontal meridia. The negative numbers label the projections of the vertical meridia in the left visual field from 30 to 150°. Meridia directly in front of ( $\pm 0^\circ$ ) and behind ( $\pm 180^\circ$ ) the fish are not shown. The overall form of the normal projection was derived from other more complete mapping experiments, including those in ref. 2. Vertical meridia in the temporal half of the right field ( $>90^\circ$ ) could not be plotted completely because of a disorderly projection. Note the pronounced compression of the nasal half of the right field ( $<90^\circ$ ) on the most anterior left tectum.

respond in any observable way to stimuli in the temporal half of the right field which would normally map on the ablated half tectum. Orientation accuracy in the remainder of the field was normal, as shown by the close match between stimulus and response angles. (Curtailing vision in one field tends to produce a small but constant bias in orientation<sup>8</sup>, seen in Fig. 1 as the vertical displacement of data points from the diagonal representing ideal orientation.) The right optic nerve was next crushed in the orbit and Fig. 1b shows that orientation was abolished throughout the right field. Within 27–30 days of optic nerve crush, orientation to stimuli in the nasal half of the right field began to return, and within 31–33 days had recovered to roughly what it had been after tectal ablation (Fig. 1c). There was then a gradual nasal-to-temporal recovery of responsiveness (Fig. 1d) which was complete by ~80 days after nerve crush, although orientation in the temporal half field was always inaccurate, even up to 420 days (Fig. 1f). In the nasal field, however, orientation generally remained accurate, with no evidence of the consistent undershooting (shown by a reduced slope) that would be expected of a compressed retinotectal map overlaying a fixed motor map. On the contrary, this particular fish showed an episode of overshooting to stimuli in the nasal field (Fig. 1e).

After collecting the behavioural data on this sunfish, the projections of both visual fields on its tectum were mapped electrophysiologically. The results have been shown in Fig. 2 by plotting certain meridia of the visual fields on to the tectal surface. Thus, the horizontal meridian seen through the left eye projects to the normal, right tectum along the line marked H, with the vertical meridia at 30° intervals plotted as shown. There is an essentially uniform mapping on the normal sunfish tectum<sup>2</sup>. All electrode positions sampled on the partially ablated left

tectum were responsive to visual stimuli somewhere in the contralateral field. The nasal half of the right field (0–90°) was represented in an orderly, but highly compressed fashion on the most anterior third of the tectum, while the remainder of the tectum received a disorganized projection from the temporal half field. In the caudal tectum, some electrode positions yielded duplicated receptive field positions, and some receptive fields mapped in reverse visuotopic order, making it impossible to plot the vertical meridia on the tectal surface.

Although only the end result of retinotectal rearrangement has been mapped in this preparation, the history of its orientation behaviour and previous results from goldfish<sup>10–12</sup> suggest the following sequence of events. Orientation in the nasal half field is quickly restored as the remaining tectum is fully innervated by fibres from the normally appropriate, temporal retina. The gradual recovery of responsiveness to temporal stimuli occurs as the caudal part of the tectal remnant is invaded by fibres from the nasal retina. However, in sunfish these foreign fibres project in a disorderly fashion, accounting for the inaccuracy of orientation in the temporal field. At the same time, the

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map of the nasal field becomes compressed into the most rostral tectum while maintaining good visuotopic order.

The behavioural findings, that accurate orientation can be performed using a greatly compressed representation of the nasal half field, and that ablation of the caudal half of the tectum does not permanently abolish the ability to orient to temporal stimuli argue strongly against the notion of a motor map fixed relative to the tectal surface. Instead, one must postulate a sensory-motor mapping of some plasticity, capable of accommodating alterations in retinotectal magnification. Normally, such an ability may be required to keep visuomotor behaviour appropriately calibrated, for during growth in fish, the visual field of each eye remains constant in extent<sup>13</sup>, while projecting uniformly on an ever-growing tectum<sup>14</sup>. The association between accuracy of orientation and orderliness of projection found in sunfish further suggests that the process of accommodation is only effective where mapping is orderly, a condition that is evidently satisfied during the accretion of retinal and tectal cells in growth.

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## Tumour promoter phorbol-12-myristate-13-acetate induces chromosomal damage via indirect action

Ingrid Emerit\* & Peter A. Cerutti†

\*Laboratory of Experimental Cytogenetics, Institut Biomédical des Cordeliers, Université Pierre et Marie Curie, 15, Rue de l'Ecole de Médecine, Paris VI, France

†Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s./Lausanne, Switzerland

The mouse skin tumour promoter phorbol-12-myristate-13-acetate (PMA) does not form covalent adducts with cellular DNA and its mutagenic potency in several systems is low or absent<sup>1–6</sup>. There have been conflicting reports of the capacity of PMA to produce sister chromatid exchanges (SCEs)<sup>3,6–8</sup>. As PMA induces the formation of superoxide radicals in polymorphonuclear leukocytes and mitogen-stimulated lymphocytes<sup>9–11</sup>, we suggested that it might produce DNA damage via indirect action by the formation of intermediate active oxygen species<sup>12,13</sup>. As is typical for other DNA-damaging agents which mainly act indirectly, for example, ionizing radiation, PMA would be expected to have low mutagenicity but high clastogenic (chromosome-breaking) activity<sup>14</sup>. In agreement with this, we report here that PMA, but not its weakly or non-promoting derivatives, induced chromosomal aberrations with high efficiency in phytohaemagglutinin (PHA)-stimulated human lymphocytes, but was only a weak producer of SCE. This activity was suppressed by superoxide dismutase, which catalyses the breakdown of superoxide radicals.

We compared the clastogenic potencies of the strong promoter PMA, the weak promoter 4-O-methyl-PMA and the non-promoter phorbol on PHA-stimulated human lymphocytes from normal donors. Table 1 shows that the clastogenic activity of these compounds paralleled their effectiveness as promoters in the mouse skin system<sup>1,2</sup>. PMA (10 ng ml<sup>-1</sup>) induced many aberrations; 4-O-methyl-PMA induced a statistically significant increase in aberrations only at a dose of 1,000 ng ml<sup>-1</sup> ( $P < 0.01$ ); and phorbol was completely inactive. The frequencies of PMA-induced aberration increased in the range 1–100 ng ml<sup>-1</sup>, but no simple dose-response relationship could be established. PMA was mitogenic in this dose range, as reported previously<sup>11</sup>.

Figure 1 shows two metaphase plates with a large number of

**Table 1** Induction of chromosomal aberrations and polyploidization in human lymphocytes by PMA, 4-O-methyl-PMA and phorbol

| Concentration<br>(ng ml <sup>-1</sup> )* | % Mitoses<br>with<br>aberrations | Aberrations<br>per 100<br>mitoses | % Poly-<br>ploidization |
|------------------------------------------|----------------------------------|-----------------------------------|-------------------------|
| <b>PMA</b>                               |                                  |                                   |                         |
| 1 (4)                                    | 13.0 ± 5.29                      | 14.0 ± 6.1                        | 6.0 ± 2.1               |
| 10 (7)                                   | 26.4 ± 7.5†                      | 33.9 ± 13.8                       | 7.7 ± 3.5†              |
| 100 (11)                                 | 33.5 ± 8.2†                      | 44.2 ± 12.9                       | 18.5 ± 5.2†             |
| <b>4-O-methyl-PMA</b>                    |                                  |                                   |                         |
| 100 (5)                                  | 10.4 ± 5.9                       | 11.6 ± 6.1                        | 0                       |
| 1,000 (5)                                | 12.9 ± 6.6‡                      | 16.0 ± 10.2                       | 0.8 ± 1.1               |
| <b>Phorbol</b>                           |                                  |                                   |                         |
| 100 (5)                                  | 7.4 ± 0.9                        | 8.2 ± 1.5                         | 0                       |
| 1,000 (5)                                | 7.8 ± 3.9                        | 7.8 ± 3.9                         | 0.8 ± 1.8               |
| Acetone 0.1% (5)                         | 7.2 ± 8.6                        | 8.0 ± 7.4                         | 1.2 ± 1.1               |
| Nil (15)                                 | 5.7 ± 2.7                        | 6.1 ± 3.1                         | 1.4 ± 1.5               |

PMA (two different lots), 4-O-methyl-PMA and phorbol (CCR, Inc., Eden Prairie, Minnesota) were dissolved in spectral grade acetone and stock solutions kept in liquid nitrogen. Appropriate aliquots of these stocks were added to whole blood or separated lymphocytes, which were cultured in Parker TC 199 medium (Flow, Zurich), supplemented with 20% human serum at the time of PHA stimulation (PHA M- and P; Difco, Geneva). The maximal concentration of acetone was 0.1%. Mitoses were blocked with colchicine after 72 h and microscopy slides prepared for chromosomal analysis according to standard procedures<sup>14</sup>. Minimally, 50 mitoses were examined for each experimental point on coded slides. Mitoses with more than five aberrations were scored as fragmentations and counted only once. They represented 1.6% of the aberrations. Values are shown ± s.d.

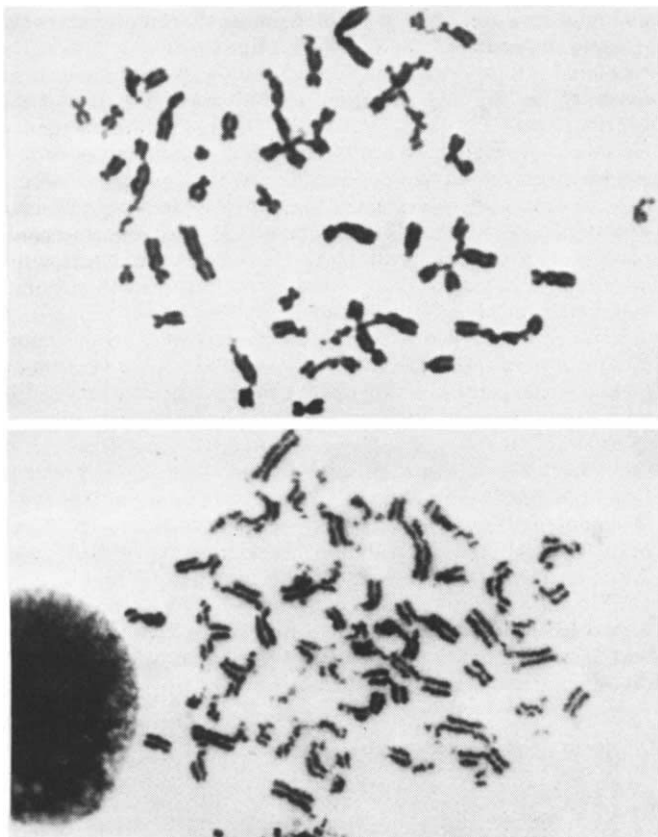
\*Values in parentheses give the number of independent experiments.

†Significance,  $P < 0.001$ .

‡Significance,  $P < 0.01$ .

PMA-induced aberrations. In 1,000 consecutive mitoses of 72-h cultures, we observed 656 chromatid breaks, 140 chromatid gaps, 71 isochromatid breaks and 34 isochromatid gaps. In addition, acentric fragments, dots, double minutes, as well as 11 tri- and quadriradial interchange figures were detected. The extent of breakage per metaphase figure varied widely from a single chromatid break to complete disintegration of the chromosomes. Atypical chromosomes were rare. Pulverization of chromosomes in diploid cells was detected in 1% of the





**Fig. 1** Two examples of mitoses with numerous chromatid breaks, minute fragments and reunion figures. These 'uncountable' aberrations were scored as fragmentations and represent 1.6% of the total number of cells with aberrations.

mitoses. Similar observations have been made previously in virus-infected cells<sup>15</sup>, after radiation or chemically induced micronucleus formation<sup>16</sup> and spontaneously in Fanconi's anaemia and Bloom's syndrome<sup>17</sup>.

PMA, but not its weakly or non-promoting derivatives, was a potent inducer of polyploidization in 72-h cultures (see Tables 1 and 2). Almost all polyploid cells were tetraploid and many showed premature chromatin condensation (PCC). All three types of PCC were present: S-phase PCC with typical pulverized or fragmented appearance of the chromatin as well as G<sub>1</sub>- and G<sub>2</sub>-phase PCC with apparently intact chromosomes having an eroded or uncoiled appearance of one or both chromatids. In certain polyploids, G<sub>2</sub>-phase PCC with strongly elongated chromosomes showed banding patterns without the use of special banding techniques (and in the absence of bromodeoxyuridine).

As shown in Table 2 the aberration frequencies increased with duration of exposure to PMA from 4 to 72 h. PMA was more effective when present during the second cell cycle (48–72 h after PHA stimulation) rather than the first (24–48 h after PHA stimulation). Lesions involving both chromatids were present at 18.6% after 72 h but only 3.3% after 48 h exposure to PMA. A few aberrations of the chromatid type were also induced when PMA was present from only 44 to 48 h after PHA stimulation, suggesting that PMA did not have to be present during S phase to induce aberrations. Cu–Zn superoxide dismutase (SOD) strongly suppressed the clastogenic activity of PMA, indicating that superoxide radicals represent intermediates in the formation of aberrations. Polyploidization was observed when PMA was present from 48 to 72 h and seems to be a result of karyokinesis without cytokinesis, rather than cell fusion. Cytopathogenic changes including induction of polyploidy after PMA treatment had been noted earlier in certain lymphoblastoid cell lines<sup>18</sup>. The mitotic index in PMA-treated cultures was increased to 3.6–5.3%, relative to 2.9% in 48-h control cultures,

due to the mitogenic potency of PMA. SOD affected neither polyploidization nor the mitotic index.

PMA was a weak inducer of SCEs in PHA-stimulated human lymphocytes. For the determination of SCE, bromodeoxyuridine (5  $\mu\text{g ml}^{-1}$  final concentration) was added to the blood cultures, which were prepared as described in Table 1 legend. Differential staining of chromatids was achieved by the method of Wolff and Perry<sup>19</sup>. The presence of 10 and 100  $\text{ng ml}^{-1}$  PMA during the 72-h culture period following PHA stimulation induced, respectively,  $7.7 \pm 3.4$  and  $8.0 \pm 2.4$  SCEs per mitosis relative to  $5.2 \pm 2.2$  SCEs per mitosis for untreated controls (significance,  $P < 0.001$ ; experimental procedures described elsewhere<sup>20</sup>). As observed for aberrations, SOD suppressed the formation of SCEs. Sister chromatid differential staining was also used to analyse the effect of PMA on the cell cycle<sup>21</sup>. At a concentration of 100  $\text{ng ml}^{-1}$  PMA had no major effect on the frequencies of cells in the first, second and third divisions (means determined 72 h after PHA stimulation: 25, 59 and 16%, respectively) relative to control cultures (22, 57 and 21%) from the same donor.

No increase in chromosomal aberrations was detected in earlier work on the induction by PMA of SCEs in Chinese hamster cells<sup>3</sup>. This could be due to the following: (1) the choice of a culture medium with a low content in radical scavengers is important for the maximal expression of chromosomal damage induced by indirect action<sup>22</sup>. (2) The pathways producing active oxygen species are particularly active in leukocytes.

We are now considering the following mechanisms for the induction by PMA of chromosomal aberrations and SCEs. (1) The active oxygen species produced in response to PMA<sup>9–11</sup> induce DNA damage by themselves or they activate a component contained in the cell or the culture media to form a clastogenic factor (CF). The generation of O<sub>2</sub><sup>-</sup> radicals in the media of lymphocyte cultures by the xanthine–xanthine oxidase system or by the photoreduction of riboflavin has been shown to result in chromosomal aberrations<sup>23</sup>. Further support for the formation of a CF by active oxygen species is derived from our work on the hereditary disease Bloom's syndrome (BS). A clastogenic factor was identified in the culture media of BS skin fibroblasts and in the serum of two BS patients; the activity of this factor could be suppressed by SOD<sup>20</sup>. (2) The burst of active oxygen species induced by PMA may induce endogenous viruses directly or by the intermediate formation of CFs. The induction of viruses by PMA is well documented<sup>18,24,25</sup> and it has been shown that PMA enhances the *in vitro* transformation of cells by adenovirus<sup>26</sup>, Epstein Barr virus<sup>27</sup> and simian virus 40 (ref. 28). Both DNA and RNA viruses can induce chromosomal damage, cell fusion and PCC<sup>16</sup>. This hypothesis presumes that lymphocytes from normal donors contain PMA-inducible endogenous viruses, but there is no evidence for this at present.

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**Table 2** PMA-induced chromosomal aberrations and polyploidization as a function of exposure time

| Exposure to PMA* (h) | Aberrations per 100 mitoses |                | % Polyploidization |                |
|----------------------|-----------------------------|----------------|--------------------|----------------|
|                      | –SOD                        | +SOD           | –SOD               | +SOD           |
| 44–48                | 10.7 $\pm$ 5.8              | 4.7 $\pm$ 1.2  | 0                  | 0              |
| 24–48                | 13.5 $\pm$ 0.9              | 5.8 $\pm$ 3.1  | 0                  | 0              |
| 0–48                 | 22.0 $\pm$ 6.0              | 8.5 $\pm$ 6.1  | 0                  | 0.7 $\pm$ 1.2  |
| 48–72                | 28.7 $\pm$ 9.9              | 10.0 $\pm$ 5.7 | 12.0 $\pm$ 6.5     | 10.0 $\pm$ 5.7 |
| 0–72                 | 33.6 $\pm$ 9.9              | 12.8 $\pm$ 5.6 | 17.6 $\pm$ 6.8     | 16.8 $\pm$ 5.8 |

Experimental conditions were as described in Table 1 legend; values are means  $\pm$  s.d. of three experiments. Bovine Cu–Zn superoxide dismutase (SOD), a gift of Dr A. M. Michelson, was added to a final concentration of 50  $\mu\text{g ml}^{-1}$ . \*PMA (100  $\text{ng ml}^{-1}$ ) present during the indicated time after PHA stimulation.

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## Consistent molecular genetic variation in human gastrointestinal carcinomas

Peter Humphries

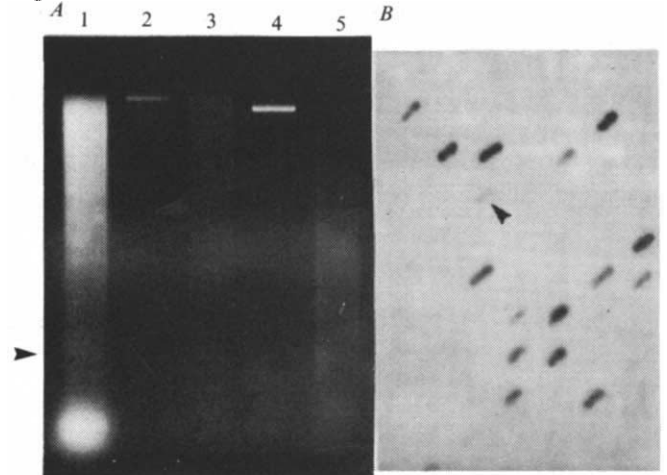
Department of Medical Genetics, Medical Biology Centre,  
Queen's University of Belfast, 97 Lisburn Road,  
Belfast BT9 7BL, UK

Investigations of chromosomal variation in human cancer have shown that malignancy is not necessarily associated with karyotypic aberration (for example, 30% of acute lymphoblastic leukaemias show no obvious changes<sup>1</sup>) and that the vast majority of alterations that do occur are of a random nature<sup>2,3</sup>. Only a few consistent chromosomal aberrations have been cytologically associated with human cancer, most notable of which are the Philadelphia translocation of chronic myelocytic leukaemia<sup>4</sup>, a deletion in chromosome 11 in Wilm's tumour<sup>5</sup> and a deletion in chromosome 13 in ~2% of retinoblastomas<sup>6</sup>. It is known that highly repetitive DNA is diminished after serial passage of human diploid fibroblasts in culture<sup>7</sup> and it is not unreasonable to suggest that loss or redistribution of genetic material could occur generally in neoplastic tissues, as proliferating tumour cells might be subjected to similar selective pressures. Here we show that, in human gastrointestinal carcinomas, the overall degeneration of DNA sequence organization as suggested by almost all cytogenetic evidence is not random and that unstable regions of the genome can be clearly distinguished from others that remain highly conserved even in advanced states of neoplasia. Quantitative assessment of such data could form the basis of an assay for early malignant transformation in gut and other human tissues.

Visual scanning of *EcoRI*-digested human DNA, separated by electrophoresis in an agarose gel (Fig. 1), reveals several discrete bands of reiterated DNA, the most prominent of which are the 340- and 680-base pair (bp) satellites<sup>7</sup>. However, there are other reiterated fragments which are largely obscured due to intense overall staining in the higher molecular-weight regions of the gel. To isolate individual DNA fragments from these higher molecular-weight regions, we excised two discrete bands of *EcoRI*-digested DNA ~14 and 50 kilobases (kb) long (Fig. 1A) and further digested these with the restriction

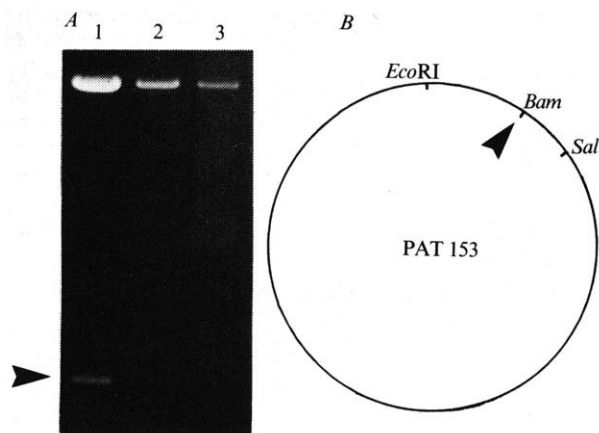
endonuclease *Sau3A*<sup>8</sup>. On cloning the smaller fragments in the plasmid vector PAT153 (ref. 9), we found that about 25% of the recombinants produced an intense autoradiographic signal in an assay of colony hybridization to <sup>32</sup>P-labelled total human genomic DNA<sup>10,11</sup> (Fig. 2B). When DNAs from these recombinants were used as hybridization probes in blot transfers of *EcoRI* digests of human genomic DNA, they produced intense overall autoradiographic smearing and no specific signals were detected (not shown). We conclude that these recombinants contain inverted repeats, the so-called middle repetitive fraction of human DNA some 200,000 of which form reiterated units widely dispersed throughout the genome<sup>12</sup>.

The rest of the recombinant colonies gave faint signals, some (arrow, Fig. 1B) arguably more intense than others. These were picked, propagated in small batches and pooled in groups of 100 to maximize the possibility of selecting plasmids carrying minor reiterated components that did not produce obvious signals in the colony hybridization to total genomic DNA. As predicted from the map (Fig. 2B), the lower molecular-weight DNA fragment of plasmid PAT153 disappeared completely as a result of the integration of 100 randomly sized pieces of human DNA. After excision from the gel of the higher molecular-weight PAT153 fragment, recombinant human DNA was electrophoretically eluted, radiolabelled with <sup>32</sup>P by nick translation<sup>13</sup> and used as a hybridization probe in blot transfers of *EcoRI* digests of normal and malignant DNA.



**Fig. 1** Construction of recombinant plasmids carrying sequences derived from 14- and 50-kb *EcoRI* fragments of human DNA. DNA was purified from human fetal liver and digested to completion with *EcoRI* restriction endonuclease, separated by electrophoresis on a 1% (w/v) agarose slab gel in a Tris/acetate buffer system and two discrete bands of DNA corresponding to ~14 and 50 kb (by reference to  $\lambda$ gtWES phage markers) were excised, electrophoretically eluted and further digested with *Sau3A*<sup>8</sup>. Conditions were chosen so as to produce partial digestion. An electrophoretic separation of these DNAs in the above conditions is shown in A. Lane 1, *EcoRI* fragments of human fetal liver DNA; lane 2, 50-kb *EcoRI* fragment; lane 3, partial *Sau3A* digest of 50-kb *EcoRI* fragment; lane 4, 14-kb *EcoRI* fragment; lane 5, partial *Sau3A* digest of 14-kb *EcoRI* fragment. The partial *Sau3A* digests of the 14- and 50-kb *EcoRI* fragments were ligated separately into the *Bam*HI site of the plasmid vector PAT153 (ref. 9). Before this, the linear plasmid was treated with bacterial alkaline phosphatase to eliminate parental recombinants<sup>10</sup>. After transformation of *E. coli* Hb101, recombinant colonies were streaked on to nitrocellulose and hybridized *in situ* to <sup>32</sup>P-labelled nick-translated total human genomic DNA (specific activity  $5 \times 10^6$  c.p.m.  $\mu\text{g}^{-1}$ ). The conditions of hybridization are described in Fig. 3 legend. After overnight incubation, hybridization filters were washed at 65 °C in  $1 \times \text{SSC}$  and then extensively in  $0.1 \times \text{SSC}$ . After exposure for 72 h, ~25% of colonies produced a distinct autoradiographic signal (B). Those not showing evidence of hybridization or giving only weak signals (arrow) were picked and grown to saturation in 10-ml batches. Cultures were pooled in groups of 100 and purified by CsCl banding and sedimentation in sucrose gradients (5-20% w/v).





**Fig. 2** Analysis of recombinant plasmids on agarose gels. **A**, wild-type plasmid PAT153 (ref. 9) (lane 1) and recombinant plasmids each containing 100 individual *Sau*3A fragments from either the 14- or 50-kb *Eco*RI fragment of fetal liver DNA (lanes 2 and 3) were purified by equilibrium centrifugation in caesium chloride, sedimented in sucrose gradients (5–20% w/v) and digested with a mixture of *Eco*RI and *Sal*I restriction endonucleases. The plasmid fragment containing the site of integration of human DNA (arrow) disappeared in recombinants. The higher-molecular-weight PAT153 DNA fragment was excised and the remaining DNA electrophoretically eluted from the agarose and used in hybridizations to normal and neoplastic DNAs. **B**, physical map of plasmid PAT153 (ref. 9) showing origin of the two fragments produced by digestion with *Eco*RI and *Sal*I restriction nucleases, and the site of integration of recombinant human DNA (arrow).

Malignant and histologically normal adjacent gut mucosal tissues were removed from five individuals, having gastrointestinal carcinomas. The DNA was digested with *Eco*RI restriction endonuclease, separated by electrophoresis in agarose slab gels (1% w/v), then blot-transferred and hybridized to <sup>32</sup>P-labelled probes prepared as above, derived from either the 14- or 50-kb *Eco*RI region of human DNA. The autoradiographs were developed for 24 h, then three of the four probes used—two from the 14-kb region and one from the 50-kb region produced signals that were almost identical. The patterns produced by one of the 14-kb-derived probes are shown in Figs 3A–D and 4A. In all cases a small number of intense, well defined signals were obtained. Band *a* of tumours T20, T22, T23 and T25 migrated at the same rate as a 14-kb  $\lambda$  phage marker and may be due either to the combined hybridization signal from the single-copy sequences in the probe, most of which would be expected to hybridize over this region, or to a reiterated component in the probe. We do not distinguish between these possibilities as they bear little significance in the interpretation of the data. The remaining signals were not caused by a single-copy human DNA fragments, as similar patterns were observed using different probes.

Tumours T20 and T22 (Fig. 3A, B), both moderately differentiated carcinomas of the sigmoid colon, produced very similar hybridization patterns, although band *f* in T22 was more intense than its counterpart in T20. Band *a* was reduced in both cases and bands *b* and *f* were almost absent in neoplastic DNAs. The weaker signal from band *c* was only partially reduced and bands *d* and *e* were reduced or absent in the tumours. Several vague bands were detected between bands *b* and *c* in mucosal samples, and band *b* itself seemed to be a composite of two hybridization signals. A diffuse area of hybridization occurred in T22 which migrated slightly faster than band *b*. This area was not present when other probes were used.

In T23 (Fig. 3C), an invasive carcinoma of the stomach, band *a* was reduced in intensity and bands *b*, *c* and *d* were almost undetectable. Band *e* in T23, which migrated at the same rate as band *d* in T20 and T22, was not reduced. Two additional faster moving bands, *f* and *g*, were either reduced or absent.

In C26 (Fig. 3D), a moderately differentiated invasive

carcinoma of the rectum, bands *a*, *e*, *h* and *i* were almost totally lost whereas bands *b*, *c*, *d*, *f* and *g* were essentially unaffected. In contrast to these observations, no differences could be detected between normal and neoplastic DNAs from T25, a carcinoma of the colon. Two major autoradiographic bands were observed which were similar to the pattern produced by two DNAs purified from the heart and lung of a human fetus (Fig. 4A). The smaller of the two bands migrated more slowly in fetal tissue.

The remaining 50-kb-derived probe produced a simpler pattern (see Fig. 4B); using this probe, only two major hybridization signals were obtained for all tumours. The first (band *a*) was at the top of the spread of *Eco*RI fragments and over the 50-kb region. These signals were probably due to the combined hybridization of single-copy sequences specific for the 50-kb *Eco*RI region, but again we do not exclude the possibility that a reiterated component of the probe may have been responsible. Band *b* has so far been detected, essentially undiminished, in all tumours. It corresponds to band *c* in T20 and T22, band *e* in T23, band *c* in C26 and band *b* in T25. We conclude that this probe contains a DNA sequence that is capable of recognizing the above bands, but sequences specific for the additional bands recognized by the other probes are absent.

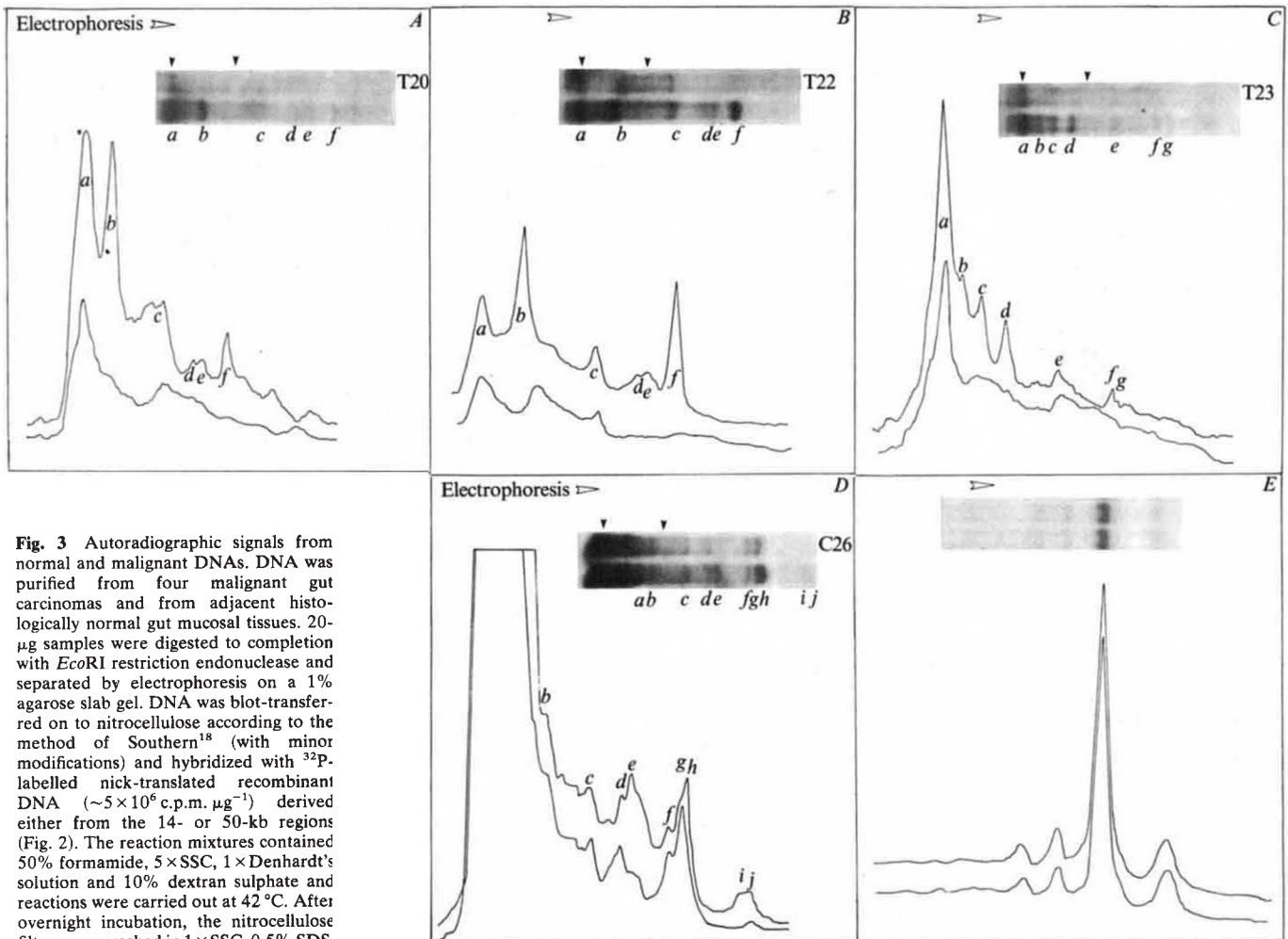
Analysis of the major *Eco*RI satellite bands in the same malignancies revealed no obvious quantitative changes (Fig. 3E). We do not, of course, exclude the possibility that changes could occur in other neoplasms.

Few molecular studies on genomic stability have been carried out on tumorigenic DNAs and none on the common human malignancies such as those described here. However, our results clearly demonstrate that consistent molecular genetic variation underlies the apparently random degeneration uniformly detected at the chromosomal level in advanced human carcinomas. The overall tendency for certain hybridization signals to disappear is almost certainly not due to loss of specific chromosomes, as hyperdiploidy and/or pseudodiploidy prevail in almost all advanced cases<sup>14</sup>. Loss of signals may reflect either the loss of the DNA sequences concerned, or their relocation to different sites within the genome.

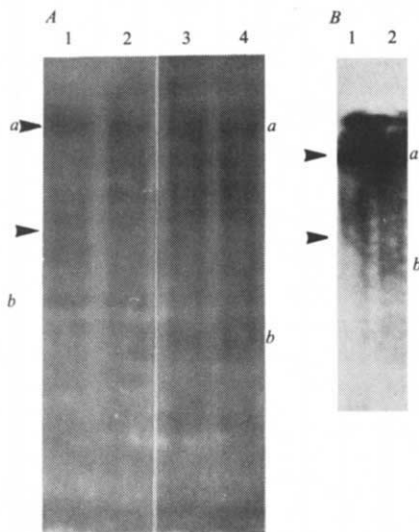
Several of the tumours, T25 and C26 in particular, produced an autoradiographic smear towards the top of the gel when *Escherichia coli* Hb101 DNA alone was used as a probe (not shown). Arguably, such smearing could overlie defined autoradiographic bands caused by sequence homologies between *E. coli* and human genomic DNA, which could have been enhanced in these experiments due to selective co-purification of specific coliform DNA fragments with fragments of human DNA after *Sal*/EcoRI digestion and electrophoretic separation. However, of the four pooled probes used, only three produced the defined signals observed in Fig. 3. With a fourth probe, derived from the 50-kb region (Fig. 4B), only one band common to the other three was observed. It therefore seems unlikely that residual levels of contaminating coliform DNA, common to all probes, produced the signals in question. We conclude that the autoradiographic smearing observed for some tumour specimens when Hb101 DNA alone was used as probe, was due to coliform contamination of these samples.

As the genomic changes observed in these experiments have been detected by comparison of normal tissues and those in advanced states of neoplasia, the question arises as to how early they start to occur and whether they are generally associated with cancer in man. Many non-heritable cancers of the type investigated here, together with the dominantly inherited familial polyposis syndromes which predispose the individual to gastrointestinal carcinoma, are often preceded by the development of alimentary polyps<sup>15</sup>. Various cytogenetic studies performed on these pre-malignancies have shown that chromosomal aberrations are generally slight<sup>16,17</sup>. Comparison of DNA changes in tissues such as these should ultimately reveal whether the changes observed here are characteristic of pre-malignancy.

In those cases where hybridization signals completely disappear, essentially all the cells in the tumour have undergone the



**Fig. 3** Autoradiographic signals from normal and malignant DNAs. DNA was purified from four malignant gut carcinomas and from adjacent histologically normal gut mucosal tissues. 20- $\mu$ g samples were digested to completion with *Eco*RI restriction endonuclease and separated by electrophoresis on a 1% agarose slab gel. DNA was blot-transferred on to nitrocellulose according to the method of Southern<sup>18</sup> (with minor modifications) and hybridized with <sup>32</sup>P-labelled nick-translated recombinant DNA ( $\sim 5 \times 10^6$  c.p.m.  $\mu$ g<sup>-1</sup>) derived either from the 14- or 50-kb regions (Fig. 2). The reaction mixtures contained 50% formamide, 5 $\times$ SSC, 1 $\times$ Denhardt's solution and 10% dextran sulphate and reactions were carried out at 42 $^{\circ}$ C. After overnight incubation, the nitrocellulose filters were washed in 1 $\times$ SSC, 0.5% SDS, then extensively in 0.1 $\times$ SSC at 65 $^{\circ}$ C. After exposure for 24 h, autoradiographs were visually scanned and photographed (A, T20; B, T22; C, T23; D, C26). Arrows on the densitometric scans indicate the approximate positions of 14- and 7-kb phage markers. In a separate experiment (E), an *Eco*RI restriction enzyme digest of human fetal liver DNA was separated by electrophoresis in a 1% agarose slab gel and the major 680-bp satellite band<sup>7</sup> excised from the gel and redigested with restriction endonuclease *Hinc*II. (For location of satellite band, see arrow in lane 1 of Fig. 1A.) After electrophoresis in a 1.5% agarose slab gel the prominent satellite fragment was excised, electrophoretically eluted and labelled with <sup>32</sup>P by nick translation. Malignant and normal mucosal DNAs were digested with *Eco*RI, separated by electrophoresis in a 1% agarose slab gel, blot-transferred and hybridized to <sup>32</sup>P-labelled satellite DNA probe. The pattern produced by tumour T23 and its normal mucosum is shown in E.



**Fig. 4** Autoradiographic signals from normal and malignant DNAs. A, lanes 1 and 2 are signals obtained using the 14-kb-derived probe of Fig. 3, from heart and lung tissue of the same fetus. Lanes 3 and 4 are signals, using the same probe, from tumour T25 and adjacent normal mucosum, respectively. Arrows indicate the positions of 7- and 14-kb phage markers. B, lanes 1 and 2 are signals obtained using the 50-kb-derived probe from tumour C26 and normal mucosal tissue, respectively. Arrows indicate origin of the DNA spread ( $\geq 50$  kb) and the position of a 7-kb phage marker.

same consistent change. If similar changes occur in malignancies of the haematopoietic system, and if they are as extensive as those already identified in gastrointestinal carcinomas, then it seems a quantitative molecular genetic means exists for the assessment of malignant cells during therapy of the acute leukaemias and other myeloproliferative disorders.

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## Expression of Ia antigen on epidermal keratinocytes in graft-versus-host disease

I. A. Lampert\*, A. J. Suitters\* & P. M. Chisholm†

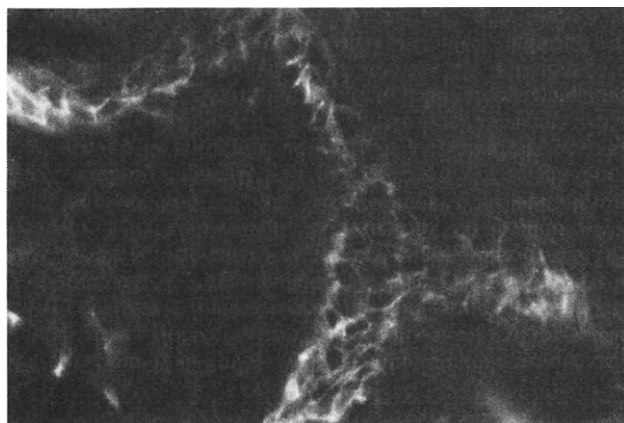
Departments of \*Histopathology and †Immunology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS, UK

The genetic information for various distinct immune functions in mice can be assigned to one or more subregions of the *I* region, which is located within the major histocompatibility complex of chromosome 17 (refs 1, 2). Cell-surface antigens encoded by genes which map within the *I* region are referred to as Ia antigens<sup>3</sup> and appear predominantly on B cells, monocytes and certain classes of macrophage, and have been described on endothelia and mitogen-stimulated T cells<sup>4</sup>. Ia antigens are also expressed on thymic epithelium<sup>5</sup> and epithelia of lactating breast<sup>6</sup> and malignant melanoma cells in culture<sup>7</sup>. Ia antigen is known to occur in the epidermis but its precise location has been controversial; available evidence has suggested that the Ia antigen is restricted to the Langerhans cell<sup>8</sup>. Here we show that it appears in keratinocytes in graft-versus-host disease.

Graft-versus-host disease was induced in 6-week-old male (PVG × PVG RT<sup>1/2</sup>) F<sub>1</sub> hybrid rats, which had been subjected to whole-body irradiation (450 rad) 24 h previously, by injection of thoracic duct lymphocytes from adult male PVG/c rats. Three doses of lymphocyte ( $25 \times 10^6$ ,  $50 \times 10^6$  and  $100 \times 10^6$ ) were given to three groups of rats. Those animals which had received  $100 \times 10^6$  lymphocytes were killed 16 days after injection; the other two groups were killed on day 20. All rats which had been given lymphocytes of the parental strain showed clinical signs of graft-versus-host disease<sup>9</sup>. Paraffin histological sections confirmed this; there was lymphoid infiltration of the epidermis and dermis, degeneration of the epidermal basal cells and dyskeratotic cells in the epidermis—all features characteristic of graft-versus-host disease<sup>9</sup>.

Frozen sections of skin from normal rats and those suffering from graft-versus-host disease were stained with mouse monoclonal anti-rat Ia antiserum MRC OX 6 (see refs 10, 11 and Fig. 1 legend) and with rabbit anti-rat Ia antiserum. In normal rat skin, staining by both anti-Ia antisera was confined to dendritic cells in the dermis, dendritic cells present in the epidermis in a suprabasilar position (presumably Langerhans cells) and in the endothelia of small blood vessels. The keratinocytes of the epidermis and hair follicles were negative. In the skins of all rats suffering from graft-versus-host disease, staining was seen in the epidermal keratinocytes, in the basal two or three layers of the epidermis and hair follicles (Fig. 1). Staining was strongest at the periphery of the cells, but was also present within the cytoplasm. In most specimens the entire length of the epidermis was stained; in others, staining was focal.

The effect of graft-versus-host disease on the Ia antigen-positive dendritic cells in the epidermis and dermis was variable. In four rats there were no Ia antigen-containing dendritic cells in the epidermis and in four others there were very few. In three rats the numbers of Ia antigen-positive dendritic cells in the dermis seemed to be increased, with apparent straddling of cells between the dermis and epidermis. In one rat the number of these cells in the dermis appeared normal and in four the numbers were reduced. In another rat, large numbers of Ia antigen-positive cells were seen in the panniculus carnosus. The cells staining in the epidermis are those regularly damaged in graft-versus-host disease, that is, the basal layers<sup>9</sup>, and the focal nature of the staining in certain specimens corresponds to the histological evidence of focal damage<sup>12</sup>. We considered whether the staining reaction was a non-specific phenomenon due to adherence of serum to damaged cells but discounted this explanation as negative results were obtained with non-specific mouse monoclonal and normal rabbit serum.



**Fig. 1** Fluorescent staining of the epidermal keratinocytes in the skin of a rat suffering from graft-versus-host disease. Note the staining of the hair follicle. Samples of skin were mounted in OCT (Ames) on a cork disk and snap-frozen in Arcton 12 (ICI) pre-cooled in liquid nitrogen. Samples were stored in liquid nitrogen. Other samples of skin were fixed in formalin and processed with paraffin for sectioning and staining with haematoxylin and eosin. Frozen sections were cut in a cryostat microtome, mounted on to glass slides, allowed to air dry and fixed in absolute ethanol for 5 min. After evaporation of the ethanol, the sections were stained with the following anti-Ia antisera. (1) Mouse monoclonal anti-rat Ia, MRC OX 6 (refs 10, 11; Sera-Labs, UK). This antibody is the product of a fusion between P3-NSI/1-Ag4-1 mouse myeloma cells and spleen cells from a BALB/c mouse immunized with rat Ia glycoprotein. The antibody binds to Ia antigens from all strains of rats tested. It binds to rat lymphocytes and 20% of thymocytes, but not to peripheral T lymphocytes. This serum was diluted in phosphate-buffered saline (PBS) to an immunoglobulin concentration of  $0.1 \text{ mg ml}^{-1}$  and incubated with the sections for 30 min. After washing, staining with monoclonal antibody was detected using FITC-conjugated goat anti-mouse antiserum (Miles). (2) Rabbit anti-rat Ia antiserum (given by Dr K. Welsh) was diluted 1/20 in PBS and sections incubated with the diluted serum as in (1). Staining due to the rabbit antiserum was detected with FITC-conjugated goat and anti-rabbit IgG (Northwest Biomedical). In (1) and (2), after washing in PBS the sections were mounted in PBS-glycerol and examined using a Leitz Ortholux UV microscope with epi-illumination. Control sera consisted of non-specific rabbit antiserum and non-specific mouse monoclonal antiserum.

The *de novo* appearance of an antigen on a cell surface raises the question of whether the antigen was synthesized by that cell or passively acquired from other cells, for example from the dendritic Ia-positive cells. No firm conclusion can be made from our results; however, the appearance of the antigen within the cytoplasm of the cell makes it probable that the antigen was synthesized by the keratinocytes themselves.

These findings are in conflict with other reports that Ia antigen in the epidermis is confined to the Langerhans cell<sup>8</sup> but are consistent with a recent study using chicken anti-human Ia antiserum in which Ia antigen in epidermal keratinocytes was seen in two of nine cases of human graft-versus-host disease (I.A.L., A.J.S., A. Thomas and G. Janossy, in preparation). It is unlikely that binding of the anti-Ia antiserum to the keratinocytes was due to cross-reactivity as the phenomenon has been described in two species for three different sera.

Other tissues can acquire Ia antigen. Normal breast epithelium is negative, whereas lactating breast is positive<sup>6</sup>; human melanoma cells acquire the antigen in culture<sup>7</sup>. The thymic epithelium, like the epidermis, is a form of stratified squamous epithelium and is positive for Ia antigen except in the case of the rudimentary embryonic thymus in immune-deficient nude mice<sup>13</sup>. The significance of these observations may become clearer when skin has been examined in a wide variety of reactions.

The Ia antigen staining of keratinocytes is unlikely to indicate a proliferation antigen, because there is a continuous process of proliferation and differentiation in the normal epidermis, which is negative. It is also unlikely that it is merely a response to

damage because Ia antigen is expressed in thymic epithelial cells in the absence of any signs of damage. It may be a response to lymphokine release by locally stimulated lymphocytes—this is supported by the finding of Ia antigen in keratinocytes in dermatoses in man (A. Thomas, personal communication). Antigens coded for by the *I* region of the major histocompatibility complex are potent stimulator antigens in graft-versus-host reactions and in mixed lymphocyte reactions<sup>14</sup>. The expression of significantly increased amounts of Ia antigen on keratinocytes during systemic graft-versus-host reaction may in part explain why the skin is a major target organ in graft-versus-host disease.

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## Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium

D. W. Mason, M. Dallman & A. N. Barclay

MRC Cellular Immunology Unit, Sir William Dunn School of Pathology, South Parks Road, Oxford OX1 3RE, UK

Ia antigens are polymorphic membrane-bound glycoproteins coded for by genes of the major histocompatibility complex. It has been shown that these genes are linked to *I*r (immune response) genes that determine the level of antibody responses made by an individual to specific antigens<sup>1</sup>. For this reason, and because of the requirement for *I*-region identity between antigen-presenting cells and T cells for their effective interaction<sup>2</sup>, Ia antigens are thought to play a part in antigen recognition by the immune system and it has been suggested that the Ia antigens are products of the *I*r genes<sup>1</sup>. In general the tissue distribution of Ia antigens is consistent with this view as they have been shown to be present on B cells, activated T cells and macrophages<sup>3</sup> and on cells other than lymphocytes in spleen, lymph node and thymus<sup>4,5</sup>. However, it has been shown that Ia antigens are also present on epithelial cells of the gut, kidney, bronchi<sup>6,7</sup> and mammary gland<sup>8</sup>. The significance of Ia antigens in tissues of nonimmune function is not understood. However, it is clear that Ia expression shows some lability in that macrophages<sup>9</sup>, T cells<sup>3</sup> and mammary gland<sup>8</sup> may all change from expressing little or no Ia to being strongly Ia-positive when appropriately stimulated. Here we show that when a systemic graft-versus-host response is induced in rats, the epidermal layer of the skin and the epithelium of the small and large intestine express large amounts of Ia antigen.

When thoracic duct lymphocytes of parental strain are injected intravenously into sublethally irradiated F<sub>1</sub> hybrid recipient rats, T cells in the donor inoculum are stimulated by the alloantigens present in the host. Because the F<sub>1</sub> recipient is genetically tolerant of the injected cells there is no concomitant reaction of the host against the injected cells. In such circumstances graft-versus-host disease is induced and proves fatal

when as few as  $3.5 \times 10^6$  cells are injected. In our experiments,  $5 \times 10^7$  parental thoracic duct lymphocytes from HO(RT1<sup>c</sup>) rats were injected into F<sub>1</sub> hybrid rats derived from crossing HO with either DA(RT1<sup>a</sup>) or HO.B2 (RT1<sup>u</sup>) inbred strains. The F<sub>1</sub> hybrid recipient rats were irradiated with a <sup>137</sup>Cs source (450 rad) a few hours before injection of parental cells. Control rats (littermates of the experimental groups) were irradiated at the same time but were injected with medium alone. The recipients of parental lymphocytes continued to gain weight and remained healthy for the first 9–10 days. After this time their skin developed a generalized erythema best seen in the ears and paws. This erythema increased for a further 2–3 days and then began to fade. The rats then began to lose weight and died in ~24 days. The control rats remained in good health and showed no skin changes. Tissues for the preparation of frozen sections were taken at day 0, that is, the day of injection, and also at days 10–12 when the skin erythema of the experimental rats was well developed.

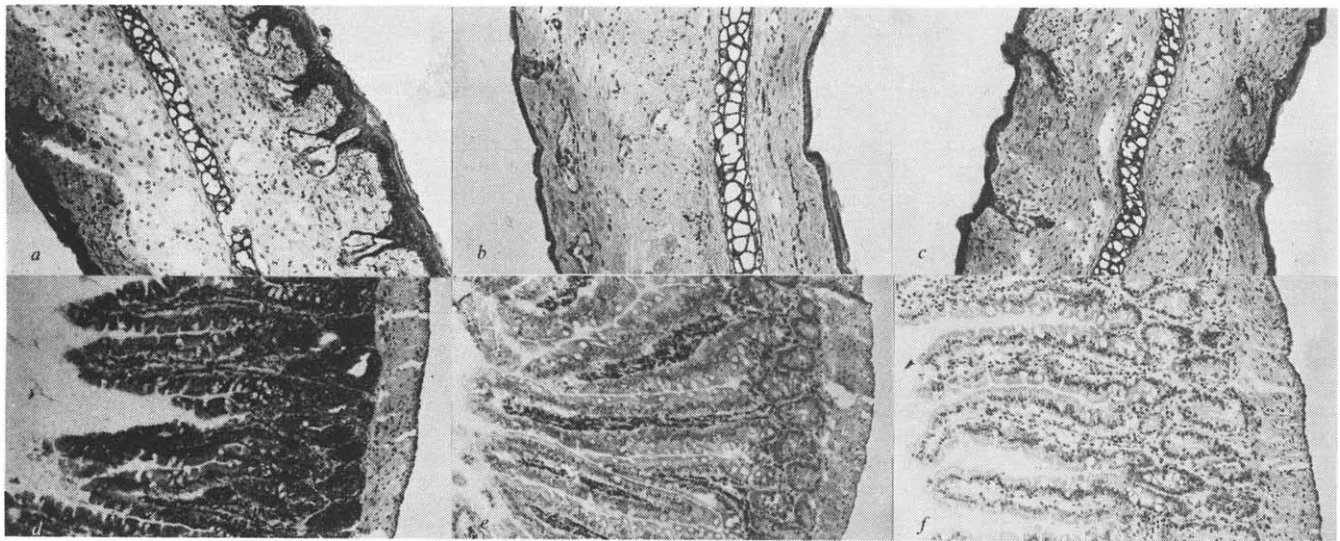
Three monoclonal mouse anti-rat Ia antibodies, MRC OX 4, MRC OX 6 and MRC OX 17, were used to detect Ia antigen in the frozen sections by an indirect immunoperoxidase technique. MRC OX 4 and MRC OX 6 recognize the rat homologue of mouse I-A antigens<sup>10</sup> and MRC OX 17 the homologue of I-E<sup>11</sup>, based on the findings that MRC OX 4 and MRC OX 6 cross-react with murine antigens that map to the I-A region and that rabbit antibodies to the rat Ia glycoprotein purified with MRC OX 17 antibody bind to mouse antigens which map, in recombinant strains, to I-E.

Figure 1a, b shows that the epidermis of the F<sub>1</sub> recipient of parental strain lymphocytes is strongly Ia-positive whereas that of the control rat, injected with medium alone, is Ia-negative. Because an identical result was obtained using MRC OX 17 antibody instead of MRC OX 6 we conclude that the rat homologues of both murine I-E and murine I-A are expressed in the epidermis of the rat receiving parental cells. Occasional Ia-positive cells were observed in the epidermis of control animals and are presumably Langerhans cells<sup>12</sup>. None of the skin sections taken on day 0 showed the generalized presence of Ia antigen in the epidermis, nor were any sections stained when a monoclonal anti-HLA (histocompatibility antigen) antibody that does not react with rat tissues was used (Fig. 1c). Figure 1d, e shows the marked increase in the level of expression of Ia antigen in the epithelium of small intestine of the rat undergoing graft-versus-host disease. Note that Ia antigen is present in small amounts in the epithelial cells covering the villi of the small intestine of normal rats<sup>13</sup> but is undetectable in the epithelium of the crypts of Lieberkühn. In contrast, as Fig. 1 shows, the crypt epithelium in the F<sub>1</sub> recipient of parental lymphocytes is strongly Ia-positive and the level of expression in the epithelium covering the villi is much increased. When frozen sections of large intestine were examined the results were similar to those obtained in the small intestine; the intestinal epithelium became Ia-positive in rats undergoing graft-versus-host disease (data not shown).

In view of the heavy labelling with monoclonal antibodies, it is unlikely that the Ia antigen observed is synthesized other than by the cells in which it is found. This is particularly so in the skin where there are few Ia-positive cells other than those found in the epidermis itself. From the uniformity of staining over individual cells and the fact that the frozen sections were thinner than the diameter of the Ia-positive cells there can be little doubt that most of the antigen detected is intracellular. It was not possible to determine from an examination of the stained sections whether or not some of the antigen was also present on the cell membrane.

The functional significance of these results is obscure. Ia expression can occur on cells that are not normally considered part of the immune system<sup>6,7</sup>; our observations agree with this conclusion. These results raise the question of the function of Ia antigens and whether they have one or more roles<sup>5</sup>. The expression of Ia in the skin and gut during graft-versus-host disease seems to be a result of the recognition of alloantigen by the





**Fig. 1** Localization of Ia antigen by an immunoperoxidase technique in epidermis and gut of rats undergoing graft-versus-host disease. *a*, Localization of Ia antigen on a cryostat section of an ear from a (HO × DA)<sub>F<sub>1</sub></sub> rat 10 days after induction of graft-versus-host disease, showing strong staining for Ia antigen in the epidermis. *b*, Control (HO × DA)<sub>F<sub>1</sub></sub> rat that had been injected with medium alone, showing complete lack of staining for Ia antigen in the epidermis. *c*, A section cut from the same block as that shown in *a* but stained with a control monoclonal anti-HLA antibody that does not react with rat tissue. Note that the keratin and cell nuclei are stained by the haematoxylin as in *b* but that the hair follicle and epidermal cells, in contrast to those in *a*, have not been labelled by the peroxidase reagent. *d*, Cryostat section of the small intestine of a (HO × HO.B2)<sub>F<sub>1</sub></sub> rat 12 days after induction of graft-versus-host disease, showing strong staining for Ia antigen in the lamina propria and in the epithelium of the villi and crypts. *e*, Control (HO × HO.B2)<sub>F<sub>1</sub></sub> rat that had been injected with medium alone, showing strong staining for Ia antigen in the lamina propria only. *f*, A section from the same block as that of *d* but stained with the control anti-HLA antibody. Graft-versus-host disease was induced in sublethally irradiated *F<sub>1</sub> recipient rats by the injection of parental strain thoracic duct lymphocytes (see text for details). On the day of injection (day 0) or on days 10–12 the tips of the ears and short lengths of small and large intestine were taken and used to prepare 5-μm cryostat sections for the localization of Ia antigen by an indirect immunoperoxidase technique<sup>15</sup>. Briefly, the sections were fixed in ethanol, washed, incubated with monoclonal antibody, washed again and then incubated with peroxidase-labelled rabbit F(ab')<sub>2</sub> anti-mouse IgG. After washing, the presence of peroxidase was revealed by incubation at room temperature with 3,3'-diaminobenzidine-HCl. To reduce nonspecific binding of the antibodies to sections of skin to negligible levels, the monoclonal antibodies (tissue culture supernatants) were diluted 1:3 with phosphate-buffered saline containing 10% bovine serum albumin and 10% normal rat serum. The same diluent was used for the peroxidase-labelled rabbit F(ab')<sub>2</sub> anti-mouse IgG reagent except that the saline concentration was increased from 0.16 M to 0.35 M.*

injected parental T cells. If it is the case that the recognition in these sites of more naturally occurring antigens by the T cells of a normal animal is also accompanied by Ia expression, then an immunological role for this expression may be anticipated. As the skin and intestinal epithelium are potential sites of entry of antigen (bronchial mucosa of rats undergoing graft-versus-host disease has not been examined for the presence of Ia antigen) it may be that the expression of Ia is an indication that, during antigen recognition, the epidermal cells of the skin and the epithelial cells of the gut take on an antigen-presenting or processing function. On the basis of this hypothesis, the presence of Ia on lactating mammary gland epithelium<sup>8</sup> might be regarded as prophylaxis against mastitis.

The observation that the epidermis and the epithelium of the small and large intestine express Ia antigen during graft-versus-host disease is of theoretical and practical significance. Graft-versus-host disease is not a pathological condition that arises outside the laboratory or hospital and it seems probable that the induction of synthesis of Ia antigen in these sites is not restricted to this disease. Expression of epidermal Ia has been reported for graft-versus-host disease in man (see accompanying paper<sup>14</sup>) and it would be of interest to determine whether local expression of Ia also occurs in chronic inflammatory or autoimmune diseases involving the skin or gut. In any event, the finding of an association between a disease and the induction of expression of Ia antigen in cells that are normally Ia-negative is likely to prove of diagnostic value.

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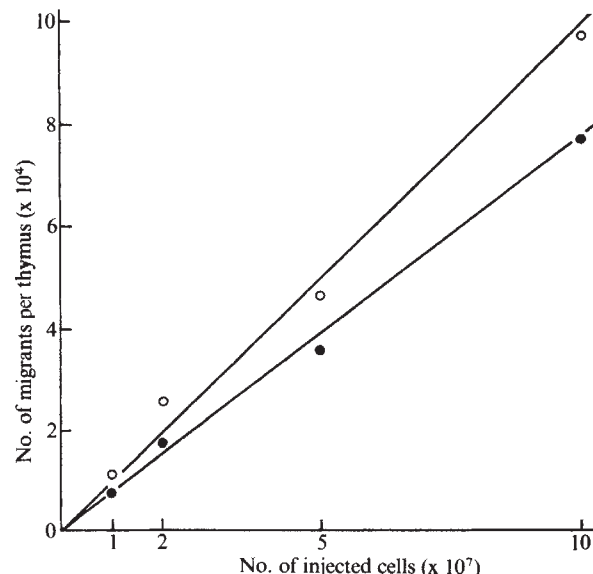
## An *in vivo* assay for thymus-homing bone marrow cells

F. Lepault\* & I. L. Weissman

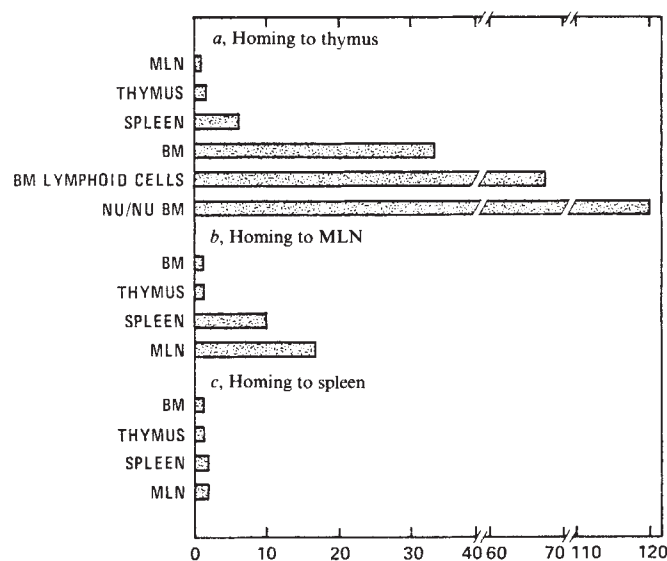
Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, California 94305, USA and \*Institut Gustave Roussy, INSERM U.66 Villejuif, France

The bone marrow of adult mammals contains a reservoir of cells capable of undergoing thymic maturation to T lymphocytes<sup>1</sup>. These 'pre-thymic' cells represent a subpopulation of bone marrow cells and have been characterized by their ability to repopulate the thymus of irradiated hosts<sup>2</sup>, by their expression of thymic markers following exposure *in vitro* to various thymic inducer polypeptides such as thymopoietin<sup>3</sup> and by their capacity to augment the response of mature T cells to mitogens<sup>4</sup>. The relationship between thymus-homing cells and 'pre-thymic' cells capable of expressing thymic surface marker and/or T-cell function has been difficult to establish because of the long assay interval in thymus-homing experiments<sup>1,2</sup>. We have now developed an *in vivo* assay for the study of thymic homing and early differentiation of direct fluorescence-labelled<sup>5</sup> bone marrow cells injected into irradiated mice. Our results show that (1) the observed migration is not random but specific, (2) the number of migrants is proportional to the number of input cells, (3) the thymus-homing bone marrow cells are not susceptible to prior *in vitro* treatment with anti-Thy-1 serum plus complement (C), and (4) the surface membrane Thy-1 antigen begins to be expressed as early as 3 h after the reconstitution.

Very early after intravenous injection of normal or irradiated hosts with fluorochrome (fluorescein isothiocyanate (FITC) or rhodamine isothiocyanate (RITC)) labelled bone marrow (BM) cells, a small number of the donor cells were detectable in the recipient thymuses. To test whether this homing was specific, we injected FITC-BM cells together with an equal number of RITC-BM, spleen, thymus or mesenteric lymph node (MLN) cells (Fig. 1). The ratio of RITC-labelled cells to FITC-labelled bone marrow cells (R/F) was determined 3–4 h later in different organs. The R/F ratio found in the recipient thymus indicates that the highest concentration of thymus-homing cells occurred in the bone marrow, and the lowest in mesenteric lymph node cells, whether normal or irradiated hosts were used. Moreover, as shown in Fig. 1a, the thymus-homing cells are contained within the bone marrow lymphoid cell fraction (enriched by Ficoll-Hypaque centrifugation) and are about fourfold greater in *nu/nu* mouse bone marrow than in normal mouse bone marrow. In fact, preliminary analysis of cloned immature thymocytes from bone marrow cells (BM-1.11) and from heterogeneous thymocyte cell suspensions (TC.3-3)<sup>6</sup> in separate experiments gave thymic homing results 5–15-fold greater than did co-injected syngeneic bone marrow cells (F. L., G. Nabel, H. Cantor and I. W., unpublished results). The homing of haematolymphoid cells to mesenteric lymph node (Fig. 1b) and to spleen (Fig. 1c) followed a completely different pattern from that of the homing to the thymus. These data suggest that the entry of the cells from the blood to the thymus was not random. In irradiated hosts the frequency of thymus-homing cells was considerably greater than that in normal recipients (data not shown) and therefore further analysis of the thymus-homing bone marrow cells was performed only in irradiated recipients.



**Fig. 2** Dose dependence of the appearance of thymus-homing bone marrow cells in the thymus of irradiated animals. The conditions of *in vitro* direct FITC labelling are described in Fig. 1 legend. Mice were killed at 3 (●) or 24 (○) h after i.v. injection of increasing doses of bone marrow cells. The FITC-BM cells which have homed to the thymus were scored as previously described<sup>16</sup> and checked for viability with propidium diiodide, which fluoresces dead cells. All immigrants excluded propidium diiodide.



**Fig. 1** Lymphoid organ homing properties of cell suspensions from haematolymphoid tissues: 4–6-week-old donors and 8–10-week-old recipients of the C57BL/6 strain were used in all experiments. Recipients were irradiated whole-body with 900 rad (250 KV X-ray machine) 2–4 h before the reconstitution. Cell suspensions from bone marrow (BM), spleen, thymus and mesenteric lymph node (MLN) were adjusted to  $5 \times 10^7$  cells per ml in 50% medium 199, 45% phosphate-buffered saline and 5% NBCS, and incubated for 20 min at 37°C with  $30 \mu\text{g ml}^{-1}$  FITC or for 15 min at room temperature with  $1 \mu\text{g ml}^{-1}$  RITC<sup>5</sup>. Cells were then spun down through a 2-cm NBCS layer, resuspended in the above medium, and  $2 \times 10^7$  FITC-BM cells injected i.v. mixed with  $2 \times 10^7$  RITC-BM, spleen, thymus or MLN cells (ratio of input RITC cells/FITC cells = 1/1). Recipients were killed 3–4 h later, and the studied organs removed. The fluorochrome-labelled cells within the cell suspensions were scored and the ratio of RITC test cells to FITC cells was calculated for each recipient organ. These ratios were normalized so that in each case, the lowest ratio was unity. The results represent the relative localization index (abscissa).

The number of the thymus-homing bone marrow cells was determined during the first 24 h after reconstitution (Fig. 2). The entry of these cells into the thymus seems to be a rapid phenomenon as only about 1.4-fold more migrants were found in the host thymus 24 h after reconstitution as at 3 h after reconstitution. The number of migrants was proportional to the number of input cells, and  $10^8$  bone marrow cells was not a saturating cell dose. Thymus-homing bone marrow cells represent  $\leq 0.1\%$  of injected cells in this assay. To determine whether these immigrants were derived from the strongly Thy-1-positive bone marrow cells ( $\sim 3\%$  of the total bone marrow cells in C57BL/6), we treated the Thy-1-positive cells before injection by incubation with well above plateau concentrations (as tested against lymph node T cells) of high-titre cytolytic monoclonal rat anti-Thy-1 antibodies of the IgM (42-21) or the IgG2a (31-11) class plus C<sup>7</sup>. Viable untreated bone marrow cells labelled with one fluorochrome were mixed with an equal number of viable anti-Thy-1-treated bone marrow cells labelled with the other fluorochrome and injected i.v. into irradiated hosts. The results (Table 1) show no evidence of detectable levels of Thy-1 on thymus-homing bone marrow cells. On the other hand, this concentration of monoclonal anti-Thy-1 (42-21) + C completely eliminates low Thy-1-bearing cells such as natural killer cells (N. Hollander, personal communication). Twenty hours after reconstitution the ratio of treated cells/standard cells was  $< 1$  (1 = ratio of the input cells). FITC fades more rapidly than RITC<sup>5</sup> and thus perhaps fewer FITC-labelled cells than RITC-labelled cells were scored at that time point. Nevertheless, the fading of the fluorescence was unselective because, regardless of the treatment of the cells, no difference between the ratio could be observed. From these experiments we conclude that the thymus-homing cells either do not bear significant levels of Thy-1 or that they are selectively resistant to cytotoxic levels of anti-Thy-1 antibodies + C. Likewise Basch and Kadish<sup>8</sup> found in long-term assays that the thymus of irradiated animals is repopulated by Thy-1-negative cells which express Thy-1 and TL antigens after *in vitro* exposure to thymopoietin<sup>9</sup>.

To test the surface antigen phenotype of thymus-homing cells, fluorochrome (usually FITC) labelled bone marrow cells were injected i.v. into irradiated hosts. The thymuses were collected



3–24 h after injection and the cell suspensions stained using indirect immunofluorescence. The second stage was either a fluorochrome (RITC if FITC was used to label the injected cells) labelled antibody to the first stage antibody, or a fluorescence labelled anti-hapten antibody. Donor cells were first identified by the initial label and the phenotype was then ascertained by monitoring the binding of the labelled antibody fluorochrome.

Preliminary experiments revealed an important artefact: host phenotype antigens such as Thy-1 and  $\kappa$  light chains were found inappropriately on injected donor cells in the thymus of the recipients during the observation interval. It seems likely that such antigens are adsorbed onto donor cells following their release from recipient irradiated cells, although other mechanisms such as unexpected expression of those markers on thymus-homing bone marrow cells has not been ruled out. As a consequence, (1) we could only test critically for donor phenotype when injections of congenic cells were used, and (2) we observed a high background when using a fluorochrome-conjugated rabbit anti-mouse antibody as second stage. It has been possible to avoid this high background by using hapten-conjugated antibodies followed by a fluorochrome-labelled anti-hapten antibody. The presence of Thy-1 antigen was monitored in C57BL/Ka-Thy-1.1 hosts injected with C57BL/Ka-Thy-1.2 congenic bone marrow cells using a rat monoclonal anti-Thy-1.2 (30-H12) and a second stage rabbit anti-rat immunoglobulin. The presence of H-2K<sup>k</sup> and I-A<sup>k</sup> antigens was determined on C57BL/6/H-2<sup>k</sup> thymus-homing bone marrow cells in C57BL/Ka (H-2<sup>b</sup>) hosts. The cells were incubated either with biotinylated mouse anti-H-2K<sup>k</sup> antibodies (clone 11-4.1) followed by rhodaminated avidin, or with nitro-iodophenyl (NIP)-conjugated mouse anti-I-A<sup>k</sup> antibodies (clone 11-5, Becton Dickinson)<sup>10</sup> as a first stage and rhodaminated anti-NIP antibodies as a second stage. The expression of TL antigens was determined by staining donor C57BL/6J/Tla<sup>a</sup> thymus-homing bone marrow cells in C57BL/Ka(Tla<sup>b</sup>) recipients.

However, the TL staining assay revealed another potential artefact: mouse monoclonal anti-TL-3 antibodies (18/20) have a IgG2a heavy chain, and we observed that this immunoglobulin subclass can bind nonspecifically to most cells in the irradiated host's thymus. For example, donor-derived cells and recipient thymocytes bind an IgG2a myeloma protein (GPC-7). This artefact has been avoided by incubating the cells with mouse IgG a few minutes before the addition of NIP-conjugated anti-TL-3 antibodies. Control staining showed that mouse IgG does not interfere with the specific binding of anti-TL-3 antibodies.

Table 2 gives the results of such experiments. Whereas donor Thy-1.2 antigens are expressed increasingly over the assay interval on thymic immigrants, over the same interval, no TL-3 positive cells were found. This is in agreement with Kamarck and Gottlieb's findings that in fetal thymus cultures the expression of Thy-1 markers appears 2–4 days before that of TL antigens<sup>11</sup>. Donor-derived H-2K<sup>k</sup> and I-A<sup>k</sup> cells were found in the thymus immigrants with the same frequency as that in the

**Table 2** Antigenic phenotype of thymus-homing bone marrow cells

| Monoclonal antibodies used  | % Thymus-homing BM cells +ve for surface antigens at different times after reconstitution |         | % Of cells positive for tested surface antigens in |          |
|-----------------------------|-------------------------------------------------------------------------------------------|---------|----------------------------------------------------|----------|
|                             | 3–4 h                                                                                     | 20–24 h | Thymus                                             | Fresh BM |
| Thy 1.2                     | 9                                                                                         | 19.5    |                                                    | 4.5      |
| Rabbit-anti-Rat Ig          | 0                                                                                         | 0       |                                                    | 1.5      |
| NIP-anti-TL + mouse Ig*     | 0                                                                                         | 0       | 58                                                 |          |
| Anti-NIP                    | 0                                                                                         | 0       | 0                                                  | 0        |
| Biotin-anti-Ia <sup>k</sup> | 4.9                                                                                       | 17.7    |                                                    | 13       |
| Avidin                      | 0                                                                                         | 0       |                                                    | 1        |
| NIP-anti-H-2K <sup>k</sup>  | 75.5                                                                                      | 90      |                                                    | 62       |
| Anti-NIP                    | 0                                                                                         | 0       |                                                    | 0        |

$2 \times 10^7$  fluorescent bone marrow cells were injected i.v. into irradiated hosts; 3–24 h later the antigenic phenotype of the fluorescent cells found in the host thymus was determined using the antibodies described in the text. Results are the mean of three to five experiments.

\*The anti-TL3 monoclonal bound nonspecifically to these cells in the absence of competing unlabelled mouse IgG. Only the anti-TL3 antibody bound nonspecifically, and only the anti-TL3 monoclonal antibody was of the IgG2a class. The unlabelled mouse immunoglobulin did not change the proportion C57BL/6J/Tla thymocytes stained by NIP-anti-TL and RITC-anti-NIP reagents (58%).

bone marrow. Thus, thymus-homing cells express high levels of H-2K<sup>k</sup> alloantigens before expressing high levels of Thy-1 during the interval assayed (Table 2). This is consistent with the ontogenetic expression of H-2 markers before that of Thy-1 markers on early thymic stem cells *in situ* or in thymus grafts, as described by Ritter<sup>12</sup> and by Owen and Raff<sup>13</sup>. The high expression of H-2K markers is maintained in outer cortical primitive lymphoblasts in neonatal and adult thymuses, and H-2K is only diminished at a later stage in the development of cortical small thymocytes<sup>14</sup>. As shown previously, the bone marrow precursors of these thymus-homing cells do not express detectable levels of Thy-1 (Table 1). Although we have not analysed the levels of other markers on such cells, we have shown, using fluoresceinated zymosan<sup>15</sup> as a marker for phagocytic cells, that less than 5% of thymus-homing bone marrow cells belong to this lineage. This corroborates our finding that the thymus-homing cells are concentrated in the bone marrow lymphoid (and not myelomonocytic) fractions of bone marrow (Fig. 1).

These experiments demonstrate that a small but significant Thy-1-negative bone marrow cell population is thymus homing on i.v. injection, the number of immigrants being proportional to the number of input cells. Thymus-homing cells by this assay are found only in tissues which allow long-term thymic reconstitution after irradiation. Such cells are found in the thymus very soon after injection, and begin to express T-cell-specific (Thy-1) markers shortly thereafter. This assay may thus be useful for analysing thymocyte developmental lineages and processes necessary for T-cell maturation.

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**Table 1** Effect of treatment of bone marrow cells with anti-Thy-1 antibody plus C on thymus-homing bone marrow cells

| Monoclonal antibody used | Hours after injection | Ratio $\frac{\text{treated FITC-BM cells in host thymus}}{\text{standard RITC-BM cells in host thymus}}$ |               |               |
|--------------------------|-----------------------|----------------------------------------------------------------------------------------------------------|---------------|---------------|
|                          |                       | Treatment of injected test cells                                                                         |               |               |
|                          |                       | Medium                                                                                                   | C             | Antibody + C  |
| 42-21                    | 3                     | 1.1 $\pm$ 0.4                                                                                            | 1.4 $\pm$ 0.4 | 0.9 $\pm$ 0.2 |
| (IgM anti-Thy-1)         | 20                    | 0.6 $\pm$ 0.1                                                                                            | 0.8 $\pm$ 0.2 | 0.6 $\pm$ 0.2 |
| 31-11                    | 3                     | 0.9 $\pm$ 0.2                                                                                            | 0.9 $\pm$ 0.2 | 1.1 $\pm$ 0.2 |
| (IgG2a anti-Thy-1)       | 20                    | 0.5 $\pm$ 0.1                                                                                            | 0.7 $\pm$ 0.2 | 0.7 $\pm$ 0.4 |

$2 \times 10^7$  viable untreated (standard) RITC-BM cells were injected i.v. simultaneously with  $2 \times 10^7$  viable FITC-BM cells treated with medium, C or anti-Thy-1 plus C in the same conditions (ratio FITC cells/RITC cells = 1/1). The number of fluorescent cells in thymocyte suspensions was scored 3 and 20 h later as previously described. The data represent the mean  $\pm$  s.e. of the ratio of FITC-test (treated) bone marrow cells/RITC-standard bone marrow cells from three to five experiments. Both the Thy-1 antibodies and the rabbit source of C were used at maximally lytic concentrations.

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## Human chromosome isolation from short-term lymphocyte culture for flow cytometry

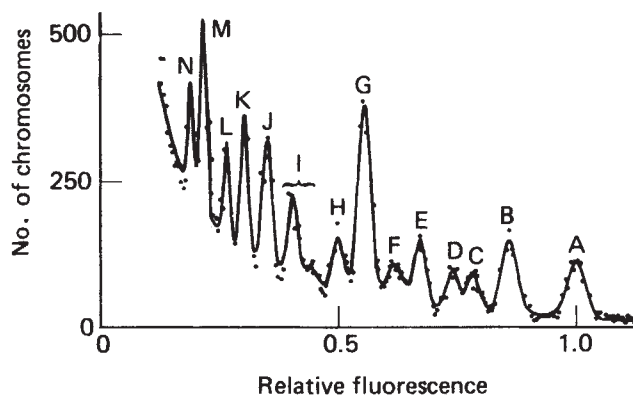
Loh-Chung Yu, Jacob Aten\*, Joe Gray & Anthony V. Carrano

Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, PO Box 5507, Livermore, California 94550, USA

Flow cytometry is uniquely suited for distinguishing between isolated mammalian chromosomes differing in DNA content<sup>1,2</sup> or DNA-base composition<sup>3,4</sup>. Using this technique, the 24 types of human chromosomes can be resolved into 15 groups when stained with Hoechst 33258 (refs 3, 5). When Hoechst 33258 (HO) and chromomycin A3 (CA3) are used, 20 groups of human chromosomes can be distinguished by dual-beam flow cytometry<sup>3,6</sup>, and the relative frequency of chromosomes in each group estimated. Flow measurements provide a precise description of the average chromosome complement of the cell population (a flow karyotype<sup>7</sup>) that is sensitive to chromosomal rearrangements<sup>2,8</sup> and frequency changes; and also enable individual chromosomes to be purified, for example, for gene mapping<sup>9</sup>. Until now, chromosomes have been isolated for flow cytometry only from established fibroblast cultures. However, the maintenance of such cultures is time-consuming and expensive, and there is the risk that chromosomal rearrangements may occur during extended fibroblast culture. Here we describe two procedures for the isolation from short-term lymphocyte cultures of metaphase chromosomes that are morphologically intact, suitable for flow cytometric analysis and purification, and have DNA of high molecular weight.

The amount of whole blood required and the time required for culture depend on the experimental objectives. For example, a flow karyotype has been obtained for chromosomes isolated from lymphocytes from 2 ml of whole blood cultured for less than 2 days. However, as much as 12 ml of whole blood may be required to produce sufficient chromosomes for biochemical or cytochemical analysis. The culture method for 12 ml of whole blood is described below.

Human blood (12 ml) obtained by venipuncture was placed in a sterile bottle containing 120 units of heparin (1,000 units per ml; Lipo-Hepin, Ricker Laboratories, California), and mixed with an equal volume of Hank's balanced salt solution (HBSS). The mixture (24 ml) was layered over 18 ml of lymphocyte separation medium (LSM) (Litton Bionetics) in a 50-ml plastic centrifuge tube and centrifuged at 400g for 30 min at room temperature. The visible white blood cell layer was collected and resuspended in seven times its volume of HBSS containing 1% fetal calf serum (FCS). The cell suspension, containing primarily lymphocytes, was then concentrated by centrifugation at 340g for 10 min. After removing all except 1.5 ml of the supernatant, the cells were resuspended and transferred to a 15-ml centrifuge



**Fig. 1** The fluorescence distribution (flow karyotype) of chromosomes isolated from cultured human lymphocytes according to method A and stained with the DNA-specific dye Hoechst 33258 ( $2 \mu\text{g ml}^{-1}$ ). Peaks a-n correspond to those listed in Table 1. The nuclei were removed by centrifugation before flow cytometry. Two methods were used for chromosome isolation. In method A,  $\sim 1-5 \times 10^6$  cells were resuspended in 0.5 ml of 75 mM KCl containing  $0.037 \mu\text{g ml}^{-1}$  Colcemid per  $10^6$  cells. The suspension was incubated at  $4^\circ\text{C}$  for  $\sim 10$  min and then centrifuged at 200g for 10 min. The cell pellet was resuspended in 0.5 ml of a Tris-based isolation buffer<sup>2</sup> containing Colcemid ( $0.037 \mu\text{g ml}^{-1}$ ). This suspension was incubated in a water bath at  $37^\circ\text{C}$  for 10 min and then placed on ice for 30 min. The chromosomes were released from the cells by passing the suspension through a 22-gauge needle one or more times. In method B, the same number of cells was resuspended in 0.5 ml of 75 mM KCl and incubated at room temperature for 10 min. A 0.3-ml solution containing 75 mM KCl, 1% Triton X-100, and  $0.2 \text{ mg ml}^{-1}$  4'-aminomethyl-4,5',8-trimethylpsoralen hydrochloride (HRI Associates, California) was then added. After a 3-min incubation, the cells were passed twice through a 22-gauge needle to release the chromosomes. The chromosome suspension was then stabilized by adding 1/4 volume of Tris-based isolation buffer concentrated five times. Cell lysis and chromosome release were monitored continuously by fluorescence microscopy after drawing a mixture of chromosomes and Hoechst 33258 (final concentration  $\sim 5 \mu\text{g ml}^{-1}$ ) by capillary action on to microslides (0.1-mm path length, Vitro Dynamics). After isolation, the chromosomes were stained with various concentrations of the DNA-specific dye Hoechst 33258 and analysed using a flow cytometer equipped with an argon-ion laser (Spectra Physics model 171).

tube, which was filled to the 15-ml mark with HBSS containing 1% FCS. This cell suspension was centrifuged again at 340g for 6 min. The cell pellet was resuspended in 4 ml of culture medium (see below). Usually 15 to 30% of the white blood cells were recovered,  $\sim 90$  to 95% of which were lymphocytes.

Lymphocyte cultures were established from this cell suspension at a density of  $2 \times 10^5$  cells per ml of culture medium. The medium consisted of RPMI 1640 (Gibco) containing 20% heat-inactivated fetal bovine serum (Gibco), 50 units  $\text{ml}^{-1}$  penicillin,  $50 \mu\text{g ml}^{-1}$  streptomycin (Flow Laboratory), 12 units  $\text{ml}^{-1}$  heparin ( $1,000$  units  $\text{ml}^{-1}$ ), 2.4 mM L-glutamine (200 mM; Gibco), and 4 ml phytohaemagglutinin-M (Gibco) per 100 ml medium. Glass prescription bottles (16-oz Sani-glass, Brockway Glass Co.) were used for the cultures (containing  $10^7$  cells per 50 ml culture medium). After  $\sim 3.5$  days, the medium was changed, and colcemid ( $0.2 \mu\text{g ml}^{-1}$ ) added to the cultures for an additional 10 h. The fraction of mitotic cells obtained from this procedure was usually about 40%. These cells were centrifuged at 200g for 10 min at  $4^\circ\text{C}$ .

This procedure can be readily adapted to isolation of chromosomes from a smaller amount of blood. For example, to culture 2 ml of human blood, the blood was mixed with 2 ml HBSS and layered over 3 ml LSM in a 15-ml plastic centrifuge tube. The lymphocytes were then purified as described above and cultured at a concentration of  $10^6$  cells per 5 ml of medium. After 45 h,  $0.2 \mu\text{g ml}^{-1}$  Colcemid was added. Ten hours later the mitotic index was between 10 and 40% and the cells were collected for chromosome isolation.

Chromosomes were isolated by two methods (see Fig. 1 legend), and analysed by flow cytometry; chromosomes were directed individually through a laser beam adjusted to emit 0.8 W at 361 and 364 nm. The resulting chromosomal fluorescence was projected through a spectral filter that transmitted

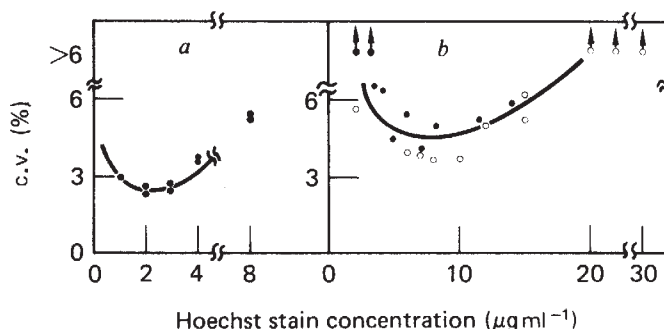
\*Permanent address: Laboratory of Radiobiology, University of Amsterdam, 121 Plesmanlaan, Amsterdam, The Netherlands.



light having wavelengths  $>450$  nm on to a photomultiplier. The pulses from the photomultiplier were then integrated, amplified, digitized, and accumulated in the memory of a pulse-height analyser to form a fluorescence distribution or flow karyotype<sup>7</sup>.

Figure 1 shows a typical flow karyotype of lymphocyte chromosomes isolated according to method A; the 24 types of human chromosomes are resolved into 15 peaks. To identify the chromosomes associated with each peak, they were sorted for quinacrine banding analysis<sup>3,5</sup>. The various chromosomes were assigned to the peaks in which they occurred most frequently (see Table 1). The measured frequencies of the larger lymphocyte chromosomes are slightly lower and the frequencies of the smaller chromosomes slightly higher than the expected frequencies. This may be due to the following: (1) some of the larger chromosomes were lost preferentially during the centrifugation to remove interphase nuclei; (2) the larger chromosomes were preferentially broken during the needle shearing; or (3) the estimates of the peak areas were biased. The results obtained for chromosomes isolated by method B were similar, although the coefficients of variation (c.v.) were slightly higher. The background observed at low fluorescence intensities is not unusual for lymphocyte chromosomes and is generally lower when the free chromosome concentration is high. The removal of nuclei by centrifugation before flow cytometry does little to reduce this background, and because the debris which constitutes the background is of a random nature, it is not a serious contaminant of chromosomes purified from this region<sup>9</sup>.

The c.v. values in the flow karyotypes of chromosomes isolated by both methods A and B, and hence the number of resolved chromosome groups, depend on the concentration of Hoechst 33258 used to stain the chromosomes (more peaks are resolved at low c.v.). We measured flow karyotypes for lymphocyte chromosomes using various concentrations of the stain to determine the concentration that produced peaks having the lowest c.v.s. Figure 2a, b shows the changes in c.v. of peak B (chromosomes 3 and 4) with dye concentration for chromosomes isolated by methods A and B, respectively. The dye concentrations that produced the lowest c.v.s were  $\sim 2 \mu\text{g ml}^{-1}$  for method A and  $\sim 8 \mu\text{g ml}^{-1}$  for method B. The lowest c.v.s ( $\sim 0.024$ ) were obtained using method A. Although the flow karyotypes obtained for chromosomes isolated using



**Fig. 2** The coefficient of variation (c.v.) of peak B in the flow karyotype as a function of the Hoechst 33258 concentration. Chromosomes were isolated according to methods A (a) and B (b). The open circles and closed circles are data points from separate experiments. Arrows indicate that the c.v.s of these samples were impossible to estimate due to poor resolution and are shown as being greater than 6%.

method B have higher c.v.s, it is a faster method, and yields more isolated chromosomes because fewer procedures are involved.

Agarose gel electrophoresis was used to determine the molecular weight of the DNA in the isolated chromosomes<sup>10</sup>. DNA samples were prepared as a chromosomal lysate<sup>11</sup> or as purified DNA, according to a modification of the Marmur method<sup>12</sup>, from a chromosomal suspension that was free of nuclei. The size markers for the molecular weight (MW) determinations were fd DNA ( $1.9 \times 10^6$  MW),  $\lambda$  DNA ( $32 \times 10^6$ ), and T4 DNA ( $120 \times 10^6$ ) (Miles Laboratories). The samples of chromosomal DNA isolated by the two methods and the marker DNAs were run in 0.6% agarose gel in Tris-borate buffer. The molecular weight of the isolated chromosomal DNA was  $>3 \times 10^7$  in all cases.

The procedures for isolating chromosomes from cultured human lymphocytes described here greatly enhance the potential of flow cytometry for population cytogenetics. We can now obtain flow karyotypes in which 14–15 groups of chromosomes are resolved; this approach may be useful for detecting chromosomal abnormalities that occur homogeneously in a large fraction of lymphocytes of an individual. The application of two-parameter flow cytometry<sup>3,4</sup> to isolated chromosomes should increase the number of resolved groups still further. The speed and quantitative nature of flow karyotyping make it especially useful for neonatal screening, evaluating abortuses and screening for inherited abnormalities<sup>2</sup>. In addition, isolation of large quantities of chromosomes directly from human peripheral lymphocytes is much less laborious than the isolation of a similar number of chromosomes from cultured human fibroblasts. Purified chromosomes can now be obtained from almost any individual thereby facilitating a wide variety of biochemical studies.

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**Table 1** Identification, relative fluorescence, and relative frequency of chromosomes isolated from human lymphocytes and analysed by flow cytometry and sorting

| Peak | Chromosome identities | Purity* (%) | Mean† | Chromosome frequency‡ | Expected chromosome frequency‡ |
|------|-----------------------|-------------|-------|-----------------------|--------------------------------|
| A    | 1, 2                  | 81          | 1.00  | 3.1                   | 4                              |
| B    | 3, 4                  | 71          | 0.86  | 3.7                   | 4                              |
| C    | 5                     | 52          | 0.78  | 1.7                   | 2                              |
| D    | 6                     | 42          | 0.74  | 1.5                   | 2                              |
| E    | 7, X                  | 55          | 0.67  | 2.5                   | 5.0                            |
| F    | 8                     | 38          | 0.61  | 2.5                   |                                |
| G    | 9–12                  | 84          | 0.54  | 7.9                   |                                |
| H    | 13                    | 57          | 0.50  | 2.2                   | 2                              |
| I    | 14                    | 56          | 0.41  | 4.0                   | 4                              |
| J    | 14, 15                | 87          |       |                       |                                |
| K    | 16, 18                | 83          | 0.33  | 4.2                   | 4                              |
| L    | 17, Y                 | 77          | 0.29  | 3.5                   | 3                              |
| M    | 20                    | 62          | 0.25  | 2.2                   | 2                              |
| N    | 19, 21                | 84          | 0.20  | 5.2                   | 4                              |
|      | 22                    | 84          | 0.18  | 1.7                   | 2                              |

Chromosome identities associated with each peak were determined by banding analysis of chromosomes sorted. The means, frequencies (proportional to peak areas) and coefficients of variation (c.v.) of the chromosome peaks were estimated by fitting the sum of several normal distributions (one from each peak) and a function of the form  $f(x) = a + bx^c$  (for the background) to the fluorescence distribution ( $a$ ,  $b$  and  $c$  are free parameters adjusted during the fit, and  $x$  is the fluorescence).

\*The smaller peak between peaks H and I in Fig. 1 was sorted separately and is enriched in chromosome 14.

†Normalized so that the mean of peak A is 1.00.

‡Normalized so that the sum is 46.

# Fetal antigen expressed on mouse erythrocytes is programmed at the early precursor stage in fetal liver

G. Mouchiroud & J. P. Blanchet\*

Département de Biologie Générale et appliquée Laboratoire associé au CNRS, Université Cl. Bernard Lyon I, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne cedex, France

In most primates and a few mammals (cattle, sheep, goat), fetal erythropoiesis, characterized by predominant synthesis of fetal haemoglobin, is replaced around birth by adult erythropoiesis<sup>1-3</sup>. In mice, fetal erythrocytes all express an antigen (Ft) that disappears after birth<sup>4</sup> and can be used as a marker for studying murine fetal and adult erythropoiesis. Murine haematopoietic tissues contain pluripotent stem cells (CFU-S) which give rise to splenic colonies when infused in irradiated adults<sup>5</sup>, committed erythroid precursors (BFU-E) that give rise to large bursts *in vitro* and more mature precursors (CFU-E) which produce small colonies *in vitro*<sup>6-8</sup>. We have now studied the expression of Ft antigen on these precursors, and their ability to produce colonies of Ft antigen-positive or -negative erythrocytes. Our results show that Ft antigen is not detected on the surface of erythroid precursors. Erythrocytes from splenic colonies are also Ft negative. However, erythrocytes from bursts and colonies derived from fetal liver BFU-E and CFU-E (and not from adult bone marrow) all express the Ft antigen, demonstrating that expression of the antigen is programmed very early in the erythropoietic sequence. Moreover, we have observed that after birth a decreasing number of CFU-E are able to give rise to Ft<sup>+</sup> colonies.

The presence of Ft antigen on erythropoietic precursors from 13-day-old fetal liver grown in plasma clots (instead of methylcellulose, see Table 1 legend) was checked by immune lysis using anti-Ft serum (1:3) and rabbit complement. This treatment affected neither the number of BFU-E ( $11 \pm 2.1$  versus  $11.2 \pm 3.3$  per  $10^4$  cells with normal serum) nor that of CFU-E ( $109.4 \pm 16.0$  versus  $107.6 \pm 18.0$  per  $2 \times 10^3$  cells). Because this antiserum at higher dilution (1:20) lysed all fetal erythrocytes and because the antiserum preserves its lytic activity after incubation with fetal liver cells, we conclude that Ft antigen is not expressed at detectable density on precursors.

Fetal liver and adult bone marrow cells were cultured in methylcellulose for 60 h. CFU-E-derived cultures were then pooled and erythrocytes separated from macrophages and granulocytes on Ficoll-Paque. Erythroid bursts were picked out individually 7 days after plating. Table 1 shows that all erythrocytes from colonies from 13-day-old liver cells are Ft positive whereas those from adult cells are all negative. To test the progeny of CFU-S, fetal liver or adult marrow cells were infused into irradiated mice and macroscopic spleen colonies were individually collected on the 7th day. Whatever their origin, all erythrocytes from these colonies were Ft negative. Insufficient maturation of erythrocytes in these spleen colonies can be ruled out because colonies collected on the 13th day again show no positive reaction whereas Ft antigen is already expressed on nucleated erythroblasts in *in vitro* colonies from fetal liver cells. Moreover when cells from these splenic colonies were plated in culture for CFU-E growth, the erythrocytes produced were again negative. These results clearly demonstrate that BFU-E and CFU-E from fetal liver are already programmed to express the Ft antigen in their progeny whereas CFU-S are not. This also explains why grafting large quantities of fetal liver cells into irradiated adults produced a transient wave of Ft<sup>+</sup> erythrocytes<sup>9,10</sup>: they probably arise from these committed erythroid precursors transferred with the grafted cells.

**Table 1** Expression of Ft antigen on erythrocytes from different erythropoietic precursor cells from 13-day-old fetal liver or adult bone marrow

| Tissue            | Precursor cells |                 |                 | CFU-E in splenic colonies |
|-------------------|-----------------|-----------------|-----------------|---------------------------|
|                   | CFU-E           | BFU-E           | CFU-S           |                           |
| Fetal liver       | Ft <sup>+</sup> | Ft <sup>+</sup> | Ft <sup>-</sup> | Ft <sup>-</sup>           |
| 13 days old       | 100%<br>(3)     | 100%<br>(30)    | 100%<br>(24)    | 100%                      |
| Adult bone marrow | Ft <sup>-</sup> | Ft <sup>-</sup> | Ft <sup>-</sup> | Ft <sup>-</sup>           |
|                   | 100%<br>(3)     | 100%<br>(20)    | 100%<br>(12)    | 100%                      |

Splenic colonies were obtained from CFU-S as described by Till and McCulloch<sup>5</sup>. C57BL/6 mice were irradiated<sup>9</sup> and grafted with  $10^5$  adult bone marrow cells or  $10^6$  fetal liver cells.  $2 \times 10^5$  or  $10^4$  cells, respectively, were plated in duplicated cultures<sup>6,8</sup> in 0.8% methylcellulose with erythropoietin  $0.2 \text{ U ml}^{-1}$  for CFU-E (serum from bled and irradiated mice provided by Dr Tambourin) or  $4 \text{ U ml}^{-1}$  for BFU-E (step III, Connaught). Cells from CFU-E were collected, washed twice in phosphate-buffered saline-bovine serum albumin (PBS-BSA) 0.5% and erythroid cells isolated by centrifugation on Ficoll-Paque (Pharmacia, 900g for 30 min at  $4^\circ\text{C}$ ). Non-erythroid cells (as observed on stained smears) were retained at the interface (respective contamination was negligible): these cells were not labelled using anti-Ft serum and fluorescein-conjugated goat anti-rabbit IgG. After three washes, cells of the pellet were tested with antiserum<sup>4</sup>. Bursts were individually collected and labelled<sup>4</sup>. Splenic colonies were individually collected 7 or 13 days after grafting, washed and labelled. The nature of the splenic colony was determined by staining of smears made in the cytocentrifuge with benzidine and May-Grunwald Giesma stain. The number of determinations is given in parentheses: CFU-E, number of cultures; BFU-E, number of bursts; CFU-S, number of splenic colonies.

During normal ontogenesis, the Ft antigen progressively disappears after birth<sup>4</sup>. We therefore studied the progeny of CFU-E from fetal liver, spleen and bone marrow during this period, first examining erythrocytes from pooled cultures. Results in Table 2 clearly show that liver, spleen and marrow all produce Ft<sup>+</sup> and Ft<sup>-</sup> erythrocytes after birth. A technique was then devised to detect the Ft antigen at the colony level (see Fig. 1 legend). Most colonies were either totally positive (Fig. 1a) or totally negative (Fig. 1b). Those which show too low or too high fluorescence to be classified as positive or negative were scored as undetermined. The frequency of positive colonies (Table 3) decreases with time both in spleen and bone marrow, in close agreement with the data of Table 2. These results confirm that the commitment to fetal antigen expression is operating much earlier than at the CFU-E stage, perhaps at the BFU-E stage. A few colonies were obviously mixed (Fig. 1c). In these colonies, positive and negative erythrocytes were not randomly distributed but were grouped in 'subclones'. Such a result would be obtained if one of the daughter cells escaped the commitment during an early division of the CFU-E.

**Table 2** Ontogenic changes in the number of Ft<sup>+</sup> erythrocytes in cultures of CFU-E from liver, spleen and marrow

| Age                | Liver    | Spleen   | Marrow  |
|--------------------|----------|----------|---------|
| 13-Day-old fetus   | 100% (3) | —        | —       |
| 16-Day-old fetus   | 100% (3) | —        | —       |
| 18-Day-old fetus   | 100% (3) | 100% (2) | ND      |
| 1-Day-old newborn  | 72% (3)  | 50% (3)  | 75% (1) |
| 15-Day-old newborn | —        | 32% (1)  | 29% (1) |
| Adult              | —        | ND       | 0% (3)  |

CFU-E were cultured and cells were collected as in Table 1. Erythrocytic cells were counted in smears stained with benzidine. The frequency of Ft<sup>+</sup> cells was determined as in Table 1. As non-erythrocytic cells were not labelled by the anti-Ft serum, the percentage of Ft<sup>+</sup> erythrocytes was deduced from the values from Ft<sup>+</sup> cells and Hb<sup>+</sup> cells. The number of repetitions is given in parentheses. ND, not determined.

\* To whom correspondence should be addressed.



**Table 3** Ontogenic changes in numbers of Ft<sup>+</sup> erythroid colonies in cultures of CFU-E from fetal liver, spleen and bone marrow

|                                | Positive CFU-E |      | Negative CFU-E |      | Mixed CFU-E |     | Undetermined CFU-E |     | Total |
|--------------------------------|----------------|------|----------------|------|-------------|-----|--------------------|-----|-------|
|                                | No.            | %    | No.            | %    | No.         | %   | No.                | %   |       |
| 13-Day-old fetal liver         | 78             | 100  | 0              | 0    | 0           | 0   | 0                  | 0   | 78    |
| 4-Day-old newborn bone marrow  | 59             | 70.2 | 19             | 22.6 | 5           | 5.9 | 1                  | 1.2 | 84    |
| 14-Day-old newborn spleen      | 152            | 70.0 | 47             | 21.7 | 11          | 5.1 | 7                  | 3.2 | 217   |
| 11-Day-old newborn bone marrow | 26             | 25.0 | 69             | 66.3 | 2           | 1.9 | 7                  | 6.7 | 104   |
| 11-Day-old newborn spleen      | 21             | 23.6 | 61             | 68.5 | 2           | 2.2 | 5                  | 5.6 | 89    |
| 21-Day-old newborn bone marrow | 11             | 13.6 | 67             | 82.7 | 0           | 0   | 3                  | 3.7 | 81    |
| 21-Day-old newborn spleen      | 3              | 13.6 | 17             | 77.2 | 0           | 0   | 2                  | 9.1 | 22    |
| Adult bone marrow              | 0              | 0    | 55             | 100  | 0           | 0   | 0                  | 0   | 55    |

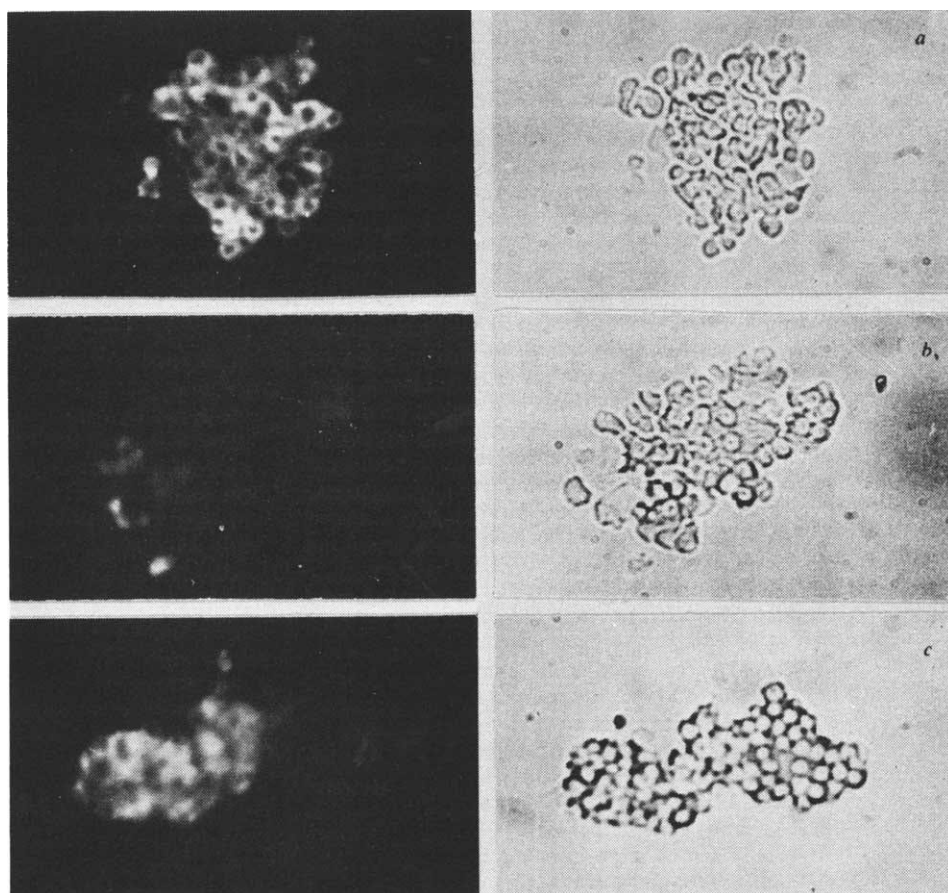
Colonies were cultured, picked out and labelled as indicated in Fig. 1 legend.

Our finding that only fetal precursors can give rise to Ft<sup>+</sup> erythrocytes in culture may be explained by two general hypotheses. First, that expression of Ft antigen is directed by accessory cells seeded together with the erythropoietic precursors. However, this is difficult to reconcile with the simultaneous detection of both Ft<sup>+</sup> and Ft<sup>-</sup> colonies in cultures of newborn precursors (Table 3). Second, that expression of Ft antigen is programmed very early. This could be explained first by the existence of two stem-cell populations (Ft<sup>+</sup> and Ft<sup>-</sup>) regulated by different inhibiting or activating factors in fetal and adult tissues. In that case, only the adult (Ft<sup>-</sup>) stem cells would give splenic colonies in irradiated adults grafted with fetal liver cells, and indeed we observed Ft<sup>-</sup> erythrocytes in these colonies. Ft<sup>+</sup> erythrocytes produced in these mice<sup>9,10</sup> would thus be progeny of the Ft<sup>+</sup>-committed erythroid precursors: this implies that the block or activation acts at the CFU-S/BFU-E transition. Alternatively, in human a single stem cell can probably give rise to both haemoglobin (Hb) F- and HbA-containing eryth-

rocytes<sup>11</sup>. If a similar situation obtains in mice, our results indicate that the decision to produce Ft<sup>+</sup> or Ft<sup>-</sup> erythrocytes occurs at the level of CFU-S/BFU-E differentiation. This determination must be relatively irreversible because production of Ft<sup>+</sup> erythrocytes from fetal erythroid precursors occurs *in vitro* and probably also *in vivo* in irradiated adults grafted with fetal liver cells<sup>9,10</sup>. Finally, production of Ft antigen could be envisaged as corresponding to the intrinsic differentiation of stem cells in fetal liver, with factors in the adult environment only directing its non-expression. This adult factor must act only on stem cells and not on erythroid precursors because we can detect some fetal erythrocytes in irradiated adults grafted with fetal liver cells<sup>9,10</sup>.

The kinetics of the disappearance of HbF in human and of Ft antigen in mice<sup>1,4</sup> and the programming of their synthesis early in erythroid differentiation (ref. 12 and present data) show strong similarities. However, there must be some difference because no Ft<sup>+</sup> erythrocytes have ever been observed in normal

**Fig. 1** Colonies obtained from 11-day-old newborn spleen cells labelled with anti-Ft antibodies. Left, UV illumination; right the same field under normal light. *a*, Positive; *b*, negative; *c*, mixed. CFU-E were cultured for 64 h at 37 °C in a humidified atmosphere of 2.5% CO<sub>2</sub> as described in Table 1 legend but methylcellulose was substituted for plasma. Red cell colonies were carefully picked out with a micropipette under an inverted microscope and incubated for 2 h at room temperature in 80 µl of eluted anti-fetal antibodies (1:3) placed in a Shandon cyto-centrifuge cell. For elution, anti-Ft antiserum (1:3) was incubated with newborn erythrocytes. After intensive washing, the antibodies were eluted with 1 M propionic acid for 2 min. After centrifugation, the supernatant was neutralized with 3 M Tris and intensively dialysed against PBS. Colonies were cyto-centrifuged on glass slides and fixed for 1 min in methanol. After soaking the slides in PBS-BSA 0.5% for 1 min one drop of goat γ-globulin anti-rabbit γ-globulin (1:25) coupled to fluorescein isothiocyanate was layered onto the colonies and kept for 30 min at 4 °C. After 2 h washing in cold PBS-BSA, a drop of rabbit γ-globulin anti-goat γ-globulin coupled to fluorescein isothiocyanate (1:25) was layered onto the slide for a further 30 min at 4 °C. After washing, the slides were examined for fluorescence. The use of a second fluorescent γ-globulin solution was rendered necessary by the low fluorescence observed after the first labelling, perhaps due to the methanol fixation of anti-Ft-treated colonies. Nevertheless, a specific membrane fluorescence was observed for colonies as well as for red blood cells (not shown).



adults or in cultures of adult precursors. The progressive disappearance of  $Ft^+$  cells and reduction of HbF synthesis seem to be best explained by a decline in the ability of the precursor cells to synthesize  $Ft$  antigen or HbF (refs 13, 14 and present data). Such switching could be assumed to be regulated, at least in part, by environmental factors acting on progenitor cells. Recent results in this field are compatible with this view<sup>15,16</sup>.

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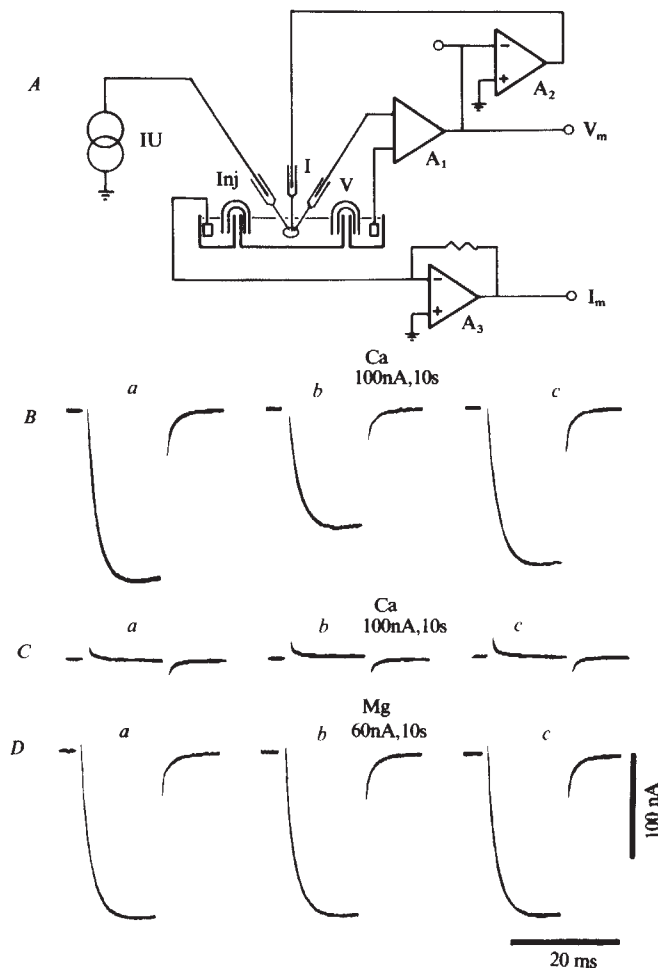
## Ca channel inactivation by intracellular Ca injection into *Helix* neurones

N. B. Standen

Department of Physiology, University of Leicester, University Road, Leicester LE1 7RH, UK

Inward calcium currents are present in many excitable tissues and are involved in several cellular processes. Depending on the tissue these may include action potential generation, transmitter release and control of membrane K permeability<sup>1,2</sup>. Membrane Ca conductance is activated by depolarization in a manner essentially similar to that described for Na conductance<sup>2,3</sup>. However, it has recently been suggested that Ca channel inactivation may differ from Na inactivation in being dependent not on membrane potential directly, but on Ca entry<sup>4–7</sup>. Such a mechanism has been proposed on the basis of the observed voltage and current dependence of Ca inactivation and could be caused either by some action of Ca ions as they pass through the channel or by an effect of increased intracellular calcium concentration  $[Ca]_i$ . I report here experiments which demonstrate that Ca inactivation may be produced directly by intracellular injection of Ca and which support the idea that Ca conductance is reduced by an increase in  $[Ca]_i$ .

Inward currents were measured under voltage-clamp from identified giant neurones of the snail *Helix aspersa*.  $SrCl_2$  (25 mM) was used in the bathing saline: Sr carries current through the Ca channel and, as inactivation is slowed in Sr, it was possible to repeat brief (<20 ms) test pulses at 10-s intervals without any cumulative inactivation developing due to the test pulses themselves. Essentially the same results were seen with external Ca instead of Sr, although more widely spaced test pulses had to be used. The saline also contained tetraethylammonium (TEA) and 4-aminopyridine (4-AP) to block outward K currents. Ca ions were injected by iontophoresis from an intracellular micropipette containing 0.1M  $CaCl_2$  (see Fig. 1A for details). Figure 1B shows the effects of Ca injection (100 nA for 10 s) on the inward current; 10 s after injection the current was reduced (trace b) from its control value and substantial recovery had occurred after 60 s (trace c). The degree of reduction in inward current depended on the size of

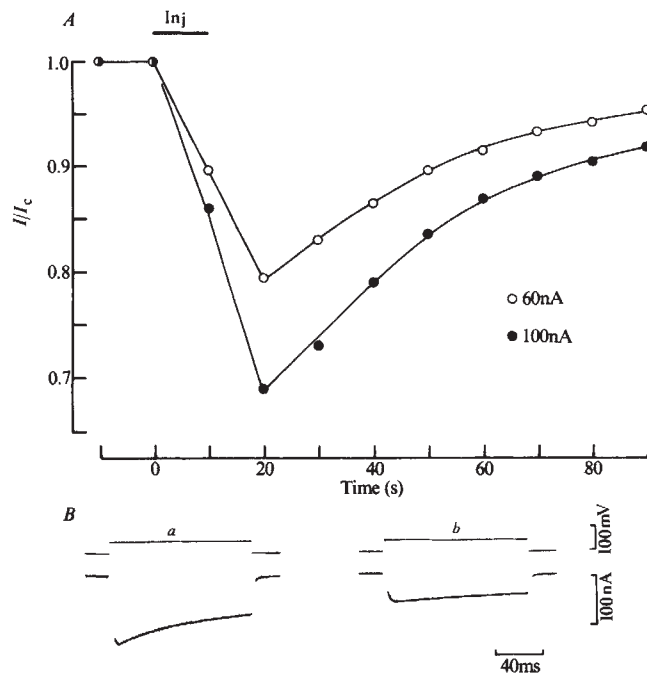


**Fig. 1** A, simplified diagram of experimental arrangement. A<sub>1</sub>, Pre-amplifier; A<sub>2</sub>, clamping amplifier; A<sub>3</sub>, virtual-earth current-to-voltage converter. IU, constant-current iontophoresis unit. Cells were impaled with three electrodes: a voltage electrode, V; a current electrode, I; and an iontophoretic injection electrode, Inj. Injections were performed with the cell clamped at the holding potential; the clamping amplifier then supplied a current equal and opposite to the injection current so that there was no membrane current flow. V<sub>m</sub>, membrane potential; I<sub>m</sub>, membrane current. B, effects of Ca injection. Traces show inward current recorded on stepping the membrane potential to 0 mV from the holding potential of -50 mV. a, Control; b, 10 s after Ca injection (100 nA, 10 s); c, 60 s after injection. C, Ca injection in the presence of 15 mM CoCl<sub>2</sub>. a, Control; b, 10 s after Ca injection (100 nA, 10 s); c, 40 s after injection. D, Mg injection. a, Control; b, 10 s after Mg injection (60 nA, 10 s); c, 40 s after injection. All these records are from the same neurone and were taken in the order D, B, C. Cell diameter 230  $\mu$ m. Temperature, 16 °C. Normal saline contained (in mM):  $SrCl_2$ , 25;  $MgCl_2$ , 5; KCl, 10; TEA Cl, 75; 4-AP, 2; Tris-HCl, 5; pH 7.5.

the injection (Fig. 2A). The effect was not an artefact of the injection itself because injections of Mg (Fig. 1D) or K had very little effect.

It seems unlikely that the reduction in inward current seen on Ca injection can be explained in terms of increased  $[Ca]_i$  causing a reduction in electrochemical driving force. Assuming that all the Ca injected remained ionized, the injection of Fig. 1B would cause  $[Ca]_i$  to rise by  $2.4 \times 10^{-4}$  M (taking the transport number for Ca to be 0.3 (ref. 8) and assuming a spherical cell with the measured diameter of 230  $\mu$ m). Using a constant field model<sup>9</sup>, a rise in  $[Ca]_i$  from  $10^{-7}$  M to  $2 \times 10^{-7}$  M would reduce the Ca current by 2%. In fact the rise in ionized  $[Ca]_i$  on injection will be much smaller than this, as the cell contains powerful mitochondrial and non-mitochondrial Ca buffer systems. Studies of Ca injection into squid axon and *Aplysia* show that >99% of injected Ca is buffered so that ionized  $[Ca]_i$  rises only a few nM per  $\mu$ M injected<sup>8,10</sup>. Therefore, in these experiments it seems





**Fig. 2** **A**, time course of Ca inactivation produced by Ca injection. Ordinate, inward current relative to control inward current ( $I/I_c$ ); abscissa, time. The duration of the injection is shown by the bar above the graph. Injection current:  $\circ$ , 60 nA;  $\bullet$ , 100 nA. Cell diameter 230  $\mu$ m. Temperature 16  $^{\circ}$ C. **B**, Ca injection in the presence of 10  $\mu$ M CCmp. Records show membrane potential (above) and membrane current (below) for a depolarizing step to 0 mV. **a**, Control; **b**, 30 s after Ca injection (50 nA, 20 s). Holding potential -50 mV. Cell diameter 260  $\mu$ m. Temperature 15  $^{\circ}$ C.

likely that the actual rise in ionized  $[Ca]_i$  is in the range  $10^{-7}$ – $10^{-6}$  M.

It could also be argued that the observed inactivation of inward current is due to a Ca-dependent outward current<sup>11,12</sup> activated by Ca injection (and insensitive to the TEA and 4-AP), giving a reduction in net inward current. If this was the case the outward current should be revealed in conditions where the inward current is blocked. Figure 1C shows that this is not the case. When inward current was blocked using 15 mM  $CoCl_2$  (substituted for  $SrCl_2$ ) there was no evidence for outward current activated by Ca injection. Similar results were obtained using 1 mM  $CdCl_2$  or in 0 Sr solution (replaced by Mg). It is also unlikely that the effects reported here result from a decrease in intracellular pH consequent on Ca injection<sup>13</sup>. First, as the Ca injections were relatively small, such pH changes would be very small ( $<0.01$  pH unit)<sup>13</sup>. Second, others have shown that EGTA injection into molluscan neurones enhances the fall in  $[pH]_i$  for a given Ca entry<sup>14</sup>, but reduces Ca current inactivation<sup>15</sup>. It seems, therefore, that Ca injection produces inactivation of Ca conductance as a result of the rise in  $[Ca]_i$ .

Figure 2A shows the time course of inactivation and recovery for Ca injections of two different sizes. Inactivation was usually maximal 5 or 10 s after a 10-s injection and recovered over the following 10 s. Such a time course is not inconsistent with Ca diffusion to the membrane if this is slowed by Ca binding; a rather similar time course of the photoresponse in certain *Aplysia* neurones has been modelled in this way<sup>16</sup>. Ca injections resulted in both greater and longer-lasting inactivation if some Ca binding was inhibited by the respiratory chain uncoupler, carbonyl cyanide *m*-chlorophenylhydrazone (CCmp), which reduces Ca uptake<sup>17</sup> in mitochondria. Figure 2B shows a typical response to Ca injection (50 nA, 20 s) in 10  $\mu$ M CCmp. Full recovery took 5–10 min in the presence of CCmp. The largest recoverable reductions in Ca inward current ( $I_{Ca}$ ) seen in these experiments were to ~30% of the control value. It might be possible to reduce  $I_{Ca}$  further using larger injecting currents; in this study the maximum injecting current was ~100 nA to avoid problems of electrode blockage.

These results show that Ca inactivation of a relatively brief time course can be produced by Ca injection. It seems likely that the mechanism involves a site on the inner membrane surface that is sensitive to  $[Ca]_i$ ; evidence for such a site has been obtained from studies of the effects of longer-term changes in  $[Ca]_i$  in dialysed cells<sup>3,18</sup>. The present results also support the hypothesis that Ca entry causes Ca channel inactivation by raising  $[Ca]_i$ <sup>4–7</sup>. Ca entering through the membrane will be much more effective at changing  $[Ca]_i$  in the region of the inner surface of the membrane than injected Ca. In studies where  $[Ca]_i$  is increased by membrane Ca entry it is often hard to separate Ca-dependent Ca inactivation from Ca-dependent K current activation<sup>2,7</sup>. It is easier to argue against the second possibility in the present case (Fig. 1C) provided that the Ca channel blockers Co and Cd do not block Ca-dependent K currents directly rather than as a consequence of blocking Ca entry: as far as I am aware no such effect has been reported.

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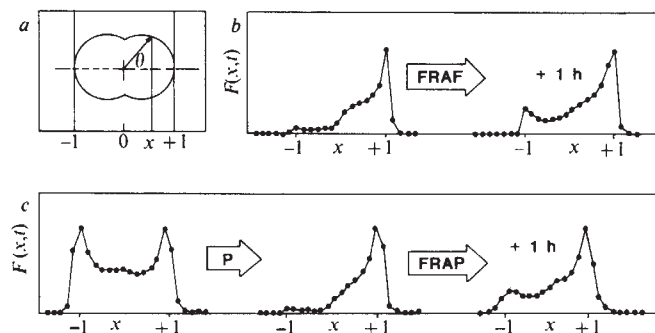
## Fluorescence photobleaching does not alter the lateral mobility of erythrocyte membrane glycoproteins

Dennis E. Koppel\* & Michael P. Sheetz†

Departments of \*Biochemistry and †Physiology, University of Connecticut Health Center, Farmington, Connecticut 06032, USA

Recently, following the pioneering experiments of Juan Yguerabide (personal communication), a fluorescence photobleaching technique has been widely used to measure lateral diffusion rates in the plane of biological membranes (for reviews see refs 1 and 2). In this technique, photobleaching of fluorescently labelled probe molecules by brief exposure to an intense laser light source is used to generate localized depletions of probe molecules. Diffusion coefficients are determined from the kinetics of fluorescence redistribution after photobleaching (FRAP), monitored by an attenuated light source. Despite the widespread application of the FRAP technique, questions remain as to whether or not dye-sensitized photodamage might seriously affect the results<sup>3–6</sup>. To test for such effects, the diffusion characteristics of a membrane system should be compared with and without fluorescence photobleaching, but this is generally extremely difficult to do—an indication of the potential capabilities of the photobleaching technique. We have now devised such a test for the diffusion of integral membrane proteins in erythrocyte membranes. We report that, within experimental error, membrane photodamage does not seem to affect the results observed in this system.

Our approach to this problem is outlined schematically in Fig. 1 with examples of actual data. We used polyethylene glycol (PEG)-induced fused erythrocyte pairs (containing haemoglobin in all cases) which assume the general 'dumbbell' shape illustrated in Fig. 1a. Membrane proteins were prelabelled with



**Fig. 1** Schematic outline of the two alternative methods of measuring membrane protein diffusion in fused erythrocyte pairs: FRAF (fluorescence redistribution after fusion) and FRAP (fluorescence redistribution after photobleaching). (See text for explanation.) For comparison, all fluorescence scans,  $F(x, t)$ , have been scaled to the same maximum value. Corrections for cell geometry in the calculation of  $c(x, t)$ , the concentration profile, were carried out in the FRAP experiments using point-by-point normalizations for each post-bleach scan by  $F(x, -)$ , a pre-bleach scan, that is:  $c(x_i, t) = F(x_i, t)/F(x_i, -)$ . In the FRAF experiments, we used  $c(x_i, t) = F(x_i, t)/[F(x_i, t) + F(-x_i, t)]$ , taking advantage of the fact that  $c(x, t)$ , in this case, is always a constant plus an odd function of  $x$ , whereas the geometrical effects are symmetric about  $x = 0$ .

dichlorotriazinylaminofluorescein<sup>7</sup>, a derivative of fluorescein that forms a covalent bond with amino groups. About two-thirds of the fluorescein is bound to band 3 protein, with the remainder on glycophorin and other undetermined components<sup>8</sup>. Procedures for cell labelling and fusion have been described previously<sup>8</sup>. One set of experiments was performed on fused pairs in which only one member of the couplet was initially labelled. Figure 1b shows  $F(x, t)$ , the fluorescence intensity profile, detected for such a pair shortly after fusion, while a focused laser beam was rapidly scanned along the long axis. (The apparatus used to measure  $F(x, t)$  is described elsewhere<sup>9</sup>.) The labelled cell (right side of scan) can be easily distinguished from the initially unlabelled cell (left side). The sharp peaks seen in these scans are due to the geometrical edge effect. The intermixing of surface proteins was followed as a function of time after fusion, by a series of many such scans over the course of  $\sim 1$  h. After 1 h at 37 °C there was a considerable shift of fluorescence from one cell to the other (see Fig. 1b). (This process is designated FRAF—fluorescence redistribution after fusion.)

A series of parallel experiments was performed on fused pairs of labelled cells using an intense laser beam to bleach the label on one of the cells. This is illustrated in Fig. 1c for a fused pair (from left to right) before bleaching, immediately after photobleaching (P) and after 1 h of fluorescence redistribution (FRAP) at 37 °C. Note that the fluorescence profile after photobleaching by design is very similar to that observed initially for the fused pair in the FRAF experiment.

In both cases, the extent of redistribution as a function of time is quantified by calculating  $\hat{\mu}_1(t)$ , the experimental estimate of the distribution asymmetry<sup>10</sup>,

$$\mu_1(t) = \frac{\int_{-1}^1 x c(x, t) dx}{\int_{-1}^1 c(x, t) dx} \quad (1)$$

where  $x$  is the cosine of equatorial angle  $\theta$  (see Fig. 1), and  $c(x, t)$  is the concentration of fluorophore on the cell surface.  $c(x, t)$  was calculated from  $F(x, t)$ , correcting for the effects of cell geometry by dividing by the values of a fluorescence scan corresponding to uniform concentration (see Fig. 1). For diffusion on the surface of a sphere, each labelled component contributes an exponential term to  $\mu_1(t)$ , with a relaxation rate proportional to the diffusion coefficient<sup>10</sup>.  $\mu_1(t)$  is not amenable to simple analysis for the complex geometry of the fused cell pair; however it does provide a means of rigorous comparison.

Figure 2 shows semilog plots of average values of  $\hat{\mu}_1(t)$  calculated for both sets of experiments, averaging the results

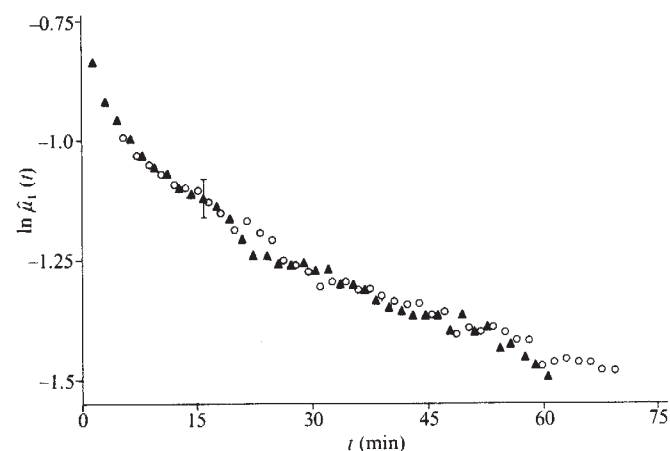
from a total of 14 different pairs of fused cells. For the FRAF data,  $t = 0$  corresponds to the time of photobleaching. The first two to three time points after bleaching reveal a rapid recovery phase not observed in the FRAF data. This difference is not unexpected, however, as the initial recovery after fusion in the FRAF experiments would be missed during the few minutes it takes to locate and position an appropriate fused pair. The agreement is seen to be excellent.

We also evaluated the long-term diffusion characteristics of the cells, and the possible residual effects of the PEG treatment used to induce fusion. In the first case, we determined whether possible photo-induced cross-linking would introduce a truly immobile component to the bleached cells. Lower limits on the mobile fraction, calculated from residual asymmetries measured after overnight ( $\sim 17$  h) incubations in the dark at 37 °C, were identical:  $0.89 \pm 0.04$  (8 measurements, FRAF) and  $0.87 \pm 0.05$  (8 measurements, FRAP).

The possible effects of PEG were examined by comparing the results of FRAP experiments on populations of single cells carried through the cell fusion procedures<sup>8</sup> with and without the addition of PEG. Figure 3 shows averaged values of  $\hat{\mu}_1(t)$  for several such cells, demonstrating little apparent difference between the two sets of data. The straight line corresponds to an average diffusion coefficient<sup>10</sup> of  $3.8 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$ .

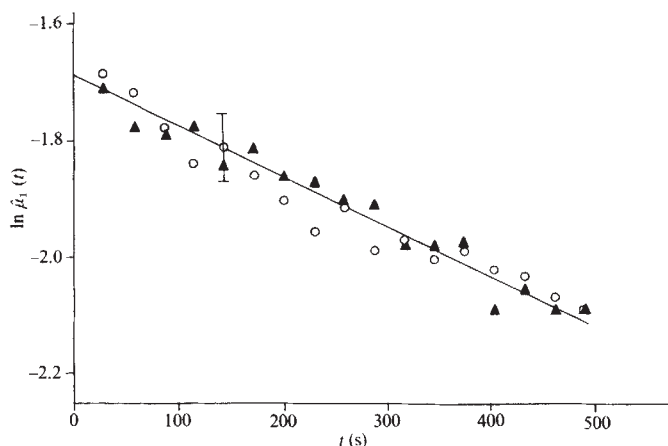
We have reported previously<sup>3</sup> that laser irradiation of fluorescein dye bound to the external surface of resealed erythrocyte membranes can, in certain circumstances, lead to extensive protein cross-linking. At the same time, we observed that the extent of photodamage decreased when a given dose of light was applied in a shorter time with a higher intensity. An extrapolation of this effect to the higher intensities ( $\sim 10^3 \text{ W cm}^{-2}$ ) and shorter exposure times ( $\sim 0.2$  s) used in the FRAP experiments reported here might account for our results. In addition, we observed that reducing agents will prevent or rapidly reverse protein cross-linking. The erythrocytes studied here were unlysed and would therefore be expected to contain  $\sim 1$  mM of reduced glutathione. Thus if oxidative cross-linking did occur, the reduced glutathione might be expected to reverse it.

The question of photodamage due to heating has been raised<sup>11</sup> and the haemoglobin-containing erythrocyte provides a critical test of that possibility. Haemoglobin absorbs at the laser wavelength (476.5 nm), so the bleaching beam would be expected to heat the cells. As we observed neither immediate lysis nor a change in the diffusion rate of membrane proteins, we suggest that heating was not a problem. We would expect even less



**Fig. 2** Semilog plots of averaged values of  $\hat{\mu}_1(t)$  calculated for both sets of experiments: O, FRAF and A, FRAP. The FRAF data are shifted relative to the FRAP  $t = 0$  by the estimated average time elapsed between fusion and the first scan ( $\sim 5$  min), maximizing the overlap between the two sets of data. The error bar corresponds to a typical standard deviation calculated for the random scatter of data, normalizing out the systematic variation from run to run in the  $t = 0$  intercept. All data were collected at 37 °C.





**Fig. 3** Semilog plots of averaged values of  $\ln f_1(t)$  for FRAP experiments on single erythrocytes at 37°C with ( $\blacktriangle$ ) and without ( $\circ$ ) PEG pretreatment. The error bar corresponds to a typical standard deviation calculated for the random scatter of data, normalizing out the systematic variation from run to run in the  $t = 0$  intercept.

heating in other cells which do not absorb at the wavelength of the bleaching beam<sup>11</sup>.

There have been other reports<sup>4,5</sup> of the absence of membrane damage effects during photobleaching experiments. Those studies, however, used the FRAP technique itself as a control, looking for changes induced by additional bleaching; thus they are subject to the argument that significant systematic error is introduced, but that it is saturated even at the minimum bleaching levels. The experiments described here rule out such an interpretation for FRAP experiments on the erythrocyte membrane.

Wey *et al.*<sup>12</sup> have recently demonstrated that the diffusion characteristics of rhodopsin in rod outer segments determined in fluorescence photobleaching experiments are essentially indistinguishable from the early measurements of diffusion in that system, based on rhodopsin absorption bleaching<sup>13</sup>. This result is complementary to our own. In the absence of an extrinsic protein matrix, rhodopsin diffusion in the disk membrane seems to be limited by the lipid bilayer viscosity<sup>12</sup>. In contrast, band 3 diffusion in erythrocyte membranes is limited by interactions with the submembranous protein matrix<sup>14,15</sup>, as seems to be the case in most plasma membranes. Thus, both types of diffusion phenomena seem to be amenable to study by fluorescence photobleaching.

We conclude that photobleaching of intact erythrocytes in aerobic conditions does not alter the lateral diffusion characteristics of the integral membrane protein, band 3. Because of the similarities of this plasma membrane to others, we suggest that the FRAP technique may prove to be a valid means of measuring the lateral mobility of plasma membrane components in most intact cells.

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## Pheromone binding and inactivation by moth antennae

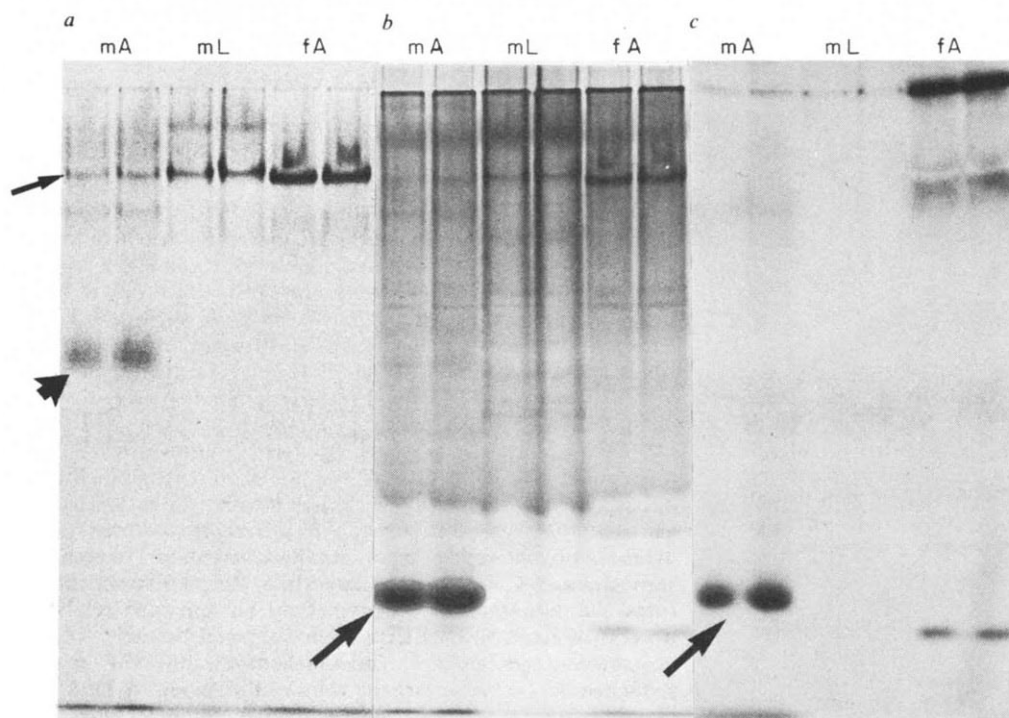
Richard G. Vogt & Lynn M. Riddiford

Department of Zoology, University of Washington, Seattle, Washington 98195, USA

The antennae of male silk moths are extremely sensitive to the female sex pheromone such that a male moth can find a female up to 4.5 km away<sup>1</sup>. This remarkable sensitivity is due to both the morphological and biochemical design of these antennae. Along the branches of the plumose antennae are the sensilla trichodea, each consisting of a hollow cuticular hair containing two unbranched dendrites bathed in a fluid, the receptor lymph<sup>2,3</sup>. The dendrites and receptor lymph are isolated from the haemolymph by a barrier of epidermal cells which secreted the cuticular hair<sup>4–6</sup>. Pheromone molecules are thought to diffuse down 100 Å-wide pore tubules through the cuticular wall and across the receptor lymph space to receptors located in the dendritic membrane<sup>6,7</sup>. To prevent the accumulation of residual stimulant and hence sensory adaptation, the pheromone molecules are subsequently inactivated in an apparent two-step process of rapid 'early inactivation' followed by much slower enzymatic degradation<sup>8,9</sup>. The biochemistry involved in this sequence of events is largely unknown. We report here the identification of three proteins which interact with the pheromone of the wild silk moth *Antheraea polyphemus*: a pheromone-binding protein and a pheromone-degrading esterase, both uniquely located in the pheromone-sensitive sensilla; and a second esterase common to all cuticular tissues except the sensilla.

[11,12-<sup>3</sup>H]trans-6,cis-11-hexadecadienyl acetate (40 Ci mmol<sup>-1</sup>; NEN) the major component of the *A. polyphemus* pheromone<sup>10</sup>, was prepared from the precursor trans-6-hexadecen-11-ynyl acetate (S. Golec and N. H. Anderson, in preparation). The labelled compound elicited similar electroantennogram responses as an unlabelled sample (supplied by Dr W. E. Roelofs). When antennal homogenates were incubated with the <sup>3</sup>H labelled pheromone for 30 min and the proteins separated by gel electrophoresis, a protein band unique to the male antennae was found to bind the pheromone (Fig. 1). This protein was neither found in other tissues (Fig. 1b) nor in the haemolymph. It was present in large amounts (~15 µg per antenna) and had an apparent molecular weight (MW) of 15,000, as determined by SDS-gel electrophoresis<sup>11,12</sup>. Minor amounts of label were associated with another rapidly migrating protein in both male and female antennae and also with proteins which remained at the top of the gel (Fig. 1c).

Several esterases were observed when gels were assayed for esterase activity using α-naphthyl acetate and β-naphthyl acetate as substrates (Fig. 1a). Two of these were of interest to us: one was associated only with the male antennae, the other with all cuticular tissues assayed (wing, head and abdominal epidermis/cuticle, and trachea) but not with brain, ventral nerve cord, fat body or haemolymph. This latter esterase from female antennae was found to bind pheromone (Fig. 1c). In this assay, the esterase specific to the male antennae metabolized β-naphthyl acetate alone whereas the integumental esterase metabolized both α- and β-naphthyl acetate. To determine whether these two esterases were capable of degrading the pheromone, antennal branch homogenates were electrophoresed and gel slices corresponding to the enzymes homogenized and incubated with excess <sup>3</sup>H-labelled pheromone. The products were separated by TLC in toluene/propanol (20:1). Both esterases degraded the pheromone ( $R_F = 0.95$ ) to the corresponding alcohol ( $R_F = 0.6$ ) with the integumental esterase showing about five times as much activity per unit tissue as the male-specific esterase. By contrast, the 15,000-MW protein, when assayed in the same way, showed no degradative activity. When the pheromone was applied directly to the wing surface, it



**Fig. 1** Comparison of proteins in homogenates of antenna and leg tissue from male and female *A. polyphemus*. Tissues were homogenized in Ephrussi-Beadle Ringer's (129 mM NaCl, 4.7 mM KCl, 1.9 mM  $\text{CaCl}_2$ ), 10 mM Tris-HCl, pH 7.0, 10% glycerol. Samples were microfuged and 100  $\mu\text{l}$  of each supernatant was incubated with  $\sim 4 \mu\text{Ci}$   $^3\text{H}$ -labelled pheromone for 45 min in Prosil-treated Corex tubes. 50  $\mu\text{l}$  of each sample were electrophoresed on a 1.5-mm thick, 12.5% native polyacrylamide slab gel<sup>17,18</sup>. The gel was stained for esterase activity<sup>19</sup> (a) or for general protein with Coomassie blue (b), then treated for 1 h in 1 M salicyclic acid for fluorography<sup>20</sup> (c). mA, male antennal branch homogenate, 12.6 mg of tissue homogenized in 300  $\mu\text{l}$  Ringer's; mL, male leg homogenate, 15 mg of tissue homogenized in 150  $\mu\text{l}$  Ringer's; fA, whole female antenna homogenate, 9.4 mg of tissue homogenized in 150  $\mu\text{l}$  Ringer's. Arrows indicate antennal-specific esterase (black arrow) and integumental esterase (grey arrow) (a) and 15,000 MW pheromone-binding protein from the male antenna (black arrow) (b, c).

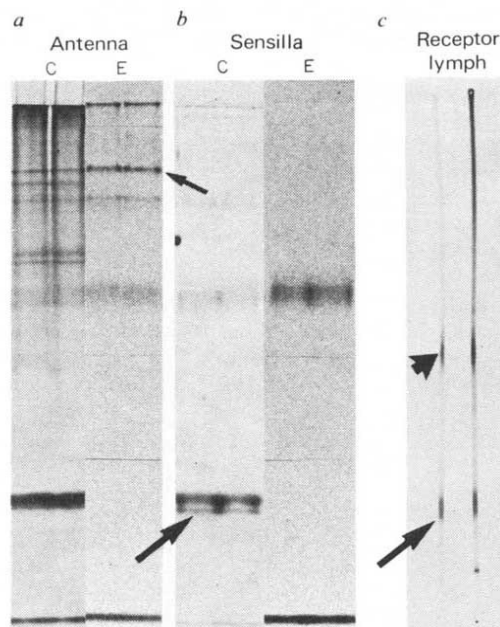
was degraded at a rate of  $4.5 \text{ pmol min}^{-1} \text{ cm}^{-2}$ . Presumably this integumental esterase hydrolyses pheromone adsorbed to the general body surface and is similar to that found in the silk moth *Bombyx mori*<sup>13</sup>.

To localize the three proteins within the male antenna, we first compared whole antennal branch homogenates with sensilla homogenates (Fig. 2a, b). Both male-specific proteins, the 15,000-MW protein and the esterase, were associated with the sensilla, but the integumental esterase was restricted to non-sensillar regions. When the receptor lymph of the sensilla was collected (by a method described elsewhere<sup>3</sup>) and analysed by microgel electrophoresis (Fig. 2c), both the male-specific esterase and the 15,000-MW protein were present.

Because the 15,000-MW protein is specific to the pheromone-sensitive sensilla, is associated with the receptor lymph and binds the sex pheromone, we subsequently refer to it

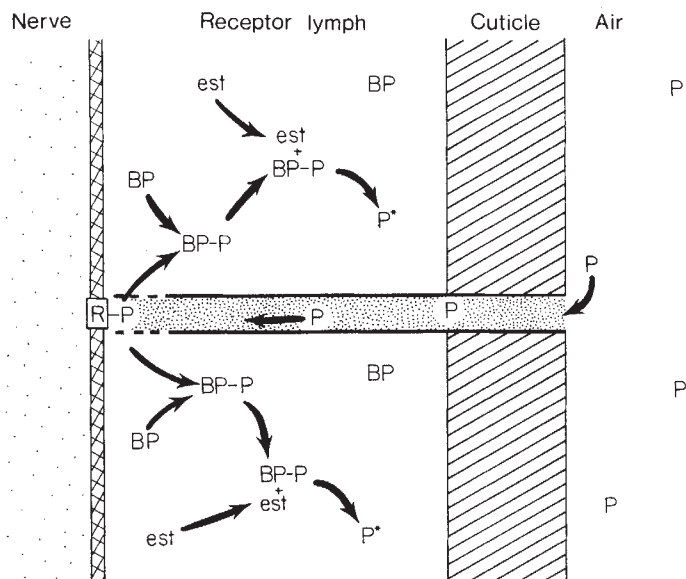
as a 'pheromone-binding protein'. Pheromone-binding proteins may be a general feature of Lepidoptera. Our comparison of male and female antennal proteins of *A. polyphemus*, *Antheraea pernyi*, *Hyalophora cecropia* and *Manduca sexta* showed that each species has a male-specific antennal protein of comparable amount and electrophoretic mobility and, in each case studied, this protein bound the *A. polyphemus* pheromone<sup>14</sup>. Furthermore, this protein has been shown to be the main protein component of the receptor lymph in *A. pernyi*<sup>3</sup>.

We suggest that both the pheromone-binding protein and the esterases play some part in the sex pheromone reception process in *A. polyphemus*. The binding protein seems an unlikely candidate for a receptor molecule because of its site in the receptor lymph, its large concentration and its apparent lack of specificity for the pheromone of the species, at least in *A. pernyi*, *H. cecropia* and *M. sexta*. We suggest, rather, that it acts



**Fig. 2** Comparison of proteins from antenna and sensilla homogenates and from receptor lymph of male *A. polyphemus*. Homogenates were prepared as described in Fig. 1 legend except that 0.005% Triton X-100 was included to ensure wetting of the hydrophobic sensilla. C, Coomassie blue stain; E, esterase stain<sup>19</sup>. a, 8 mg antennal branches were homogenized in 200  $\mu\text{l}$  Ringer's. b, Sensilla were cut from branches of 16 antennae with micro-scissors and homogenized in 2.5 ml Ringer's. Homogenates were microfuged, and the supernatant concentrated by vacuum dialysis (Prodi-Con, Bio Molecular Dynamics) to 140  $\mu\text{l}$ , then 30  $\mu\text{l}$  of 50% glycerol were added. c, Two microgels comparing receptor lymph (left) and antennal branch homogenate (right). Receptor lymph was collected from 40 branches on to the outside of a flame-sealed micro-glass electrode by the method of Kaissling<sup>3</sup> and washed into 4  $\mu\text{l}$  of Ringer's (Ephrussi-Beadle, 10 mM Tris-HCl, pH 7.0, 10% glycerol, 0.002% bromophenol blue). The entire 4  $\mu\text{l}$  was electrophoresed on a single microgel<sup>21</sup> (10% non-SDS polyacrylamide tube gel, cast to 5 cm in a 10  $\mu\text{l}$  Van-Lab micropipet with a 2-cm, 5% stacking gel) at 100  $\mu\text{A}$  constant current. The antennal branch micro-gel represents 4  $\mu\text{l}$  of 3.7 mg branches homogenized in 150  $\mu\text{l}$  Ringer's. Arrows indicate 15,000-MW pheromone-binding protein (black arrow), the antennal specific esterase (black arrowhead), and the general esterase (grey arrowhead).





**Fig. 3** A model of the part played by the pheromone-binding protein (BP) and the sensilla esterase (est) during pheromone reception in *A. polyphemus*. After interacting with the presumed receptor (R) in the dendritic membrane to initiate sensory input, the pheromone (P) is inactivated by becoming bound to a pheromone-binding protein in the receptor lymph and subsequently degraded by a sensilla esterase. The pore-tubule is represented by a broken line near the receptor, to represent the uncertainty of the nature of the pore-tubule at this point<sup>2,7</sup>. P\*, pheromone degradation product.

together with the sensilla esterase to inactivate the pheromone after it has interacted with the presumed membrane-bound receptor. In this scheme (Fig. 3), pheromone would first diffuse down a pore-tubule to the presumed receptor, located in the dendritic membrane, to initiate sensory input. The pheromone would then become inactivated in a two-step process, first binding to a pheromone-binding protein in the receptor lymph and then being degraded by a sensilla esterase. The direction and rate of this process would be favoured by the large concentration of binding protein.

Rapid clearing of residual pheromone from the sensilla is behaviourally important to the animal while following a pheromone trail as the moth must know immediately when he leaves the pheromone plume. The existence of such a process is supported by electrophysiological<sup>8,9</sup> and behavioural<sup>15,16</sup> studies. The integumental esterase would further ensure that any pheromone adsorbing to the body surface would be degraded so that the male's body would not itself become a pheromone source.

We thank Dr Simon Golec for synthesizing the precursor used in the preparation of the tritiated pheromone and Dr Wendell Roelofs for a sample of authentic *A. polyphemus* pheromone. R.G.V. was supported by NIH National Research Service Award GM07270. This study was supported by NSF grants PCM76-18800 and 80-11152 to L.M.R.

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## Evidence that $P_{fr}$ is not the active form of phytochrome in light-grown maize

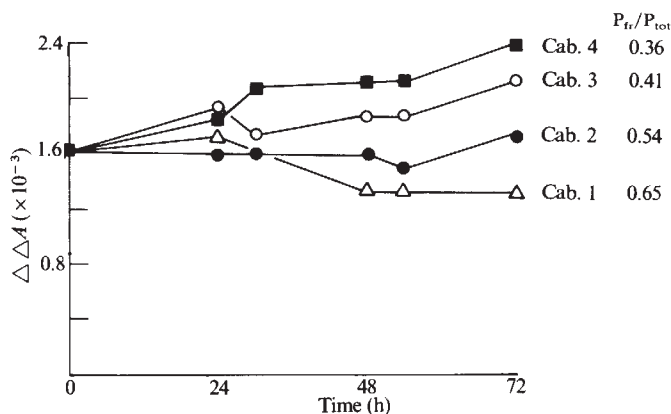
Harry Smith

Department of Botany, University of Leicester,  
Leicester LE1 7RH, UK

The biological activity of the plant photochromic photoreceptor, phytochrome, is generally thought to reside in  $P_{fr}$ , the far-red-absorbing form, whilst the red-absorbing  $P_r$  is inactive. This view is based solely on incomplete correlations between spectrophotometrically detectable  $P_{fr}$  and responses in etiolated tissues given brief light treatments<sup>1,2</sup>. Studies of light-grown seedlings have recently revealed an inverse, linear relationship between phytochrome-regulated extension growth and phytochrome photoequilibrium (defined as  $P_{fr}/(P_r + P_{fr})$  and estimated from the spectral photon distribution of the actinic radiation)<sup>3–10</sup>. Such a relationship could only fit the ' $P_r$  as active form' theory if  $P_r + P_{fr}$  (that is,  $P_{total}$ ) remained constant and independent of the light sources used. Here I report data from herbicide-bleached, light-grown maize seedlings which show that  $P_{total}$  is strongly dependent on the wavelength distribution of the radiation and that the amount of  $P_{fr}$  does not correlate with growth response.

Due to the overriding absorbance of chlorophyll, phytochrome can normally not be measured in light-grown leaves by *in vivo* spectrophotometry. The bleaching herbicide norflurazon, however, prevents chlorophyll accumulation<sup>11</sup> and has no effect on the formation of phytochrome, thereby allowing the use of *in vivo* spectrophotometry<sup>12–14</sup>. Maize seedlings, sown in and watered daily with  $5 \times 10^{-5}$  M norflurazon, were grown for 7 days in continuous white light with a red:far-red photon ratio simulating that of normal daylight, after which they were transferred for 72 h to a range of specially constructed growth cabinets<sup>15</sup> providing equal amounts of photosynthetically active radiation, but differing red:far-red photon ratios simulating various degrees of vegetational shade<sup>3,4,10</sup>. *In vivo* spectrophotometry showed that very small amounts of phytochrome were present, and in a survey of several maize varieties, a range of about  $0.4\text{--}2.5 \times 10^{-3} \Delta\Delta A_{730}^{660}$  was observed, using the standard 14-leaf assay sample (see Fig. 1 legend). To ensure that the observed red/far-red (R/FR) absorbance changes were due to phytochrome, a  $P_r$  minus  $P_{fr}$  difference spectrum was constructed and was shown to be identical to that previously published by Jabben<sup>14</sup> for norflurazon-treated maize leaves, with absorption maxima at 665 nm and 725 nm, an isosbestic point at  $\sim 685$  nm and a R:FR ratio of 1.2.

Exposure of seedlings to the four available simulated-canopy light sources on days 7–10 led to substantial changes in the amounts of  $P_{total}$  present in the second leaf. Figure 1 shows the spectrophotometrically measured phytochrome ( $P_{total}$ ) values obtained at intervals during the treatment period. At the start of this period, the mean  $P_{total}$  value was  $1.6 \times 10^{-3} \Delta\Delta A_{730}^{660}$ , and by 72 h there was almost twice as much phytochrome present in leaves treated with white light having a low red:far-red photon ratio ( $P_{fr}/P_{total} = 0.36$ ) than in identical leaves held under a high red:far-red ratio ( $P_{fr}/P_{total} = 0.65$ ). Thus, light sources establishing a low proportion of  $P_{fr}$  at photoequilibrium allow the net accumulation of phytochrome, whereas sources which establish a high proportion of  $P_{fr}$  lead to a net loss of phytochrome. These



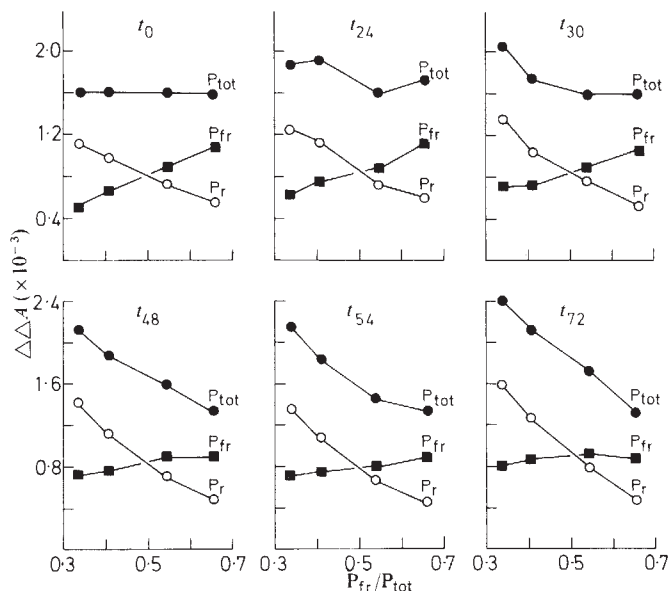
**Fig. 1** Changes in the total phytochrome content of norflurazon-treated maize second leaves following a transition in light quality. *Zea mays* L. cv. John Innes Hybrid No. 1. seeds (Asmer) imbibed for 4 h in  $5 \times 10^{-5}$  M norflurazon [(San 9789) 4-chloro-5-(methylamino)-2-( $\alpha, \alpha, \alpha$ -trifluoro-*m*-tolyl)-3(2*H*)-pyridazinone (97.5% pure)] and were watered with the same solution daily. Samples of 14 leaves, held closely pressed to each other by transparent adhesive tape, were placed, apex down, in plastic 10-mm cuvettes, opaque except for apertures for the measuring and actinic beams, and inserted in a Perkin-Elmer dual wavelength spectrophotometer. The measuring beams, set at 660 and 730 nm, were  $5 \times 1.5$  mm and traversed the leaves at ~7–12 mm from their tips. Actinic sources were set at 660 and 730 nm with Balzer interference filters, irradiating the sample from the side.  $P_{\text{total}}$  values were obtained by multiplying the  $\Delta\Delta A_{730}^{660}$  by 1.33 to account for incomplete photoconversion by the red actinic source<sup>19</sup>. Samples were taken from seedlings grown for 7 days ( $t_0$ ) in a white light cabinet (R:FR 0.6928,  $178 \mu\text{mol m}^{-2} \text{s}^{-1}$ , 400–800 nm) and at 24-h ( $t_{24}$ ,  $t_{48}$ ,  $t_{72}$ ) intervals after transfer to four cabinets, each giving equal 400–700-nm photon fluence rates ( $40 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), but having different amounts of added FR thus establishing different phytochrome photoequilibria.

observations can be explained if the phytochrome of the light-grown plant is similar to that of the dark-grown plant, in which  $P_r$  is virtually stable but  $P_{tr}$  is rapidly destroyed.

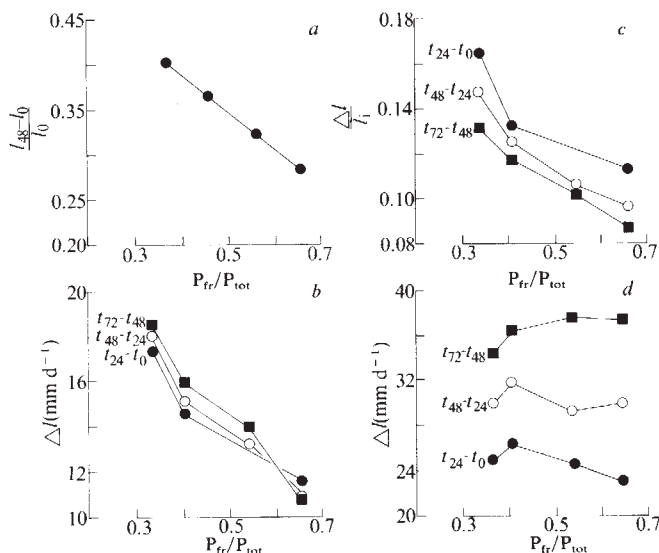
The amounts of phytochrome present were too low to allow reliable direct measurement of amounts of  $P_r$  and  $P_{tr}$  by *in vivo* spectrophotometry. Using dark-grown maize leaves, which contain ~50 times more phytochrome, the  $P_{tr}/P_{\text{total}}$  established by the four light sources was confirmed to be equivalent to the values derived from the spectral photon distributions. Consequently, it was possible to calculate the  $P_r$  and  $P_{tr}$  amounts which would be present at photoequilibrium in the norflurazon-treated maize leaves in the four cabinets from the directly measured  $P_{\text{total}}$  values.

Figure 2 shows how the levels of  $P_{\text{total}}$ ,  $P_r$  and  $P_{tr}$  change during the 3-day treatment period. The relationship between photoequilibrium and each of the three parameters changes markedly between  $t_0$  and  $t_{72}$ . Note that the amount of  $P_r$  becomes virtually independent of the red:far-red photon ratio of the light at steady-state, thus neatly confirming the prediction of Schäfer<sup>16</sup> that, at steady state under continuous irradiation,  $P_r$  concentration should become independent of wavelength.

To establish the appropriate correlations between the measured phytochrome parameters and growth rate, it is important to measure growth rate at different times during the treatment period. Owing to their limited growth capacity, it was not possible to obtain all the required data from norflurazon-treated seedlings, although it was shown that second-leaf extension during days 7–9 was inversely related in a linear manner to the  $P_{tr}/P_{\text{total}}$  established by the incident radiation (Fig. 3a). This fact is of interest in its own right, because it shows definitively that neither photosynthesis nor the photosynthetic pigments can be responsible for the observed responses to red:far-red light quality. Also, the responses to light quality in norflurazon-treated maize are qualitatively similar to those



**Fig. 2** The relationship between maize leaf phytochrome content and the estimated phytochrome photoequilibrium established by the incident radiation. The data of Fig. 1 are expressed as a function of the photoequilibrium established by the light sources.  $P_{fr}$  contents were obtained by multiplying the measured  $P_{\text{total}}$  values by the appropriate  $P_{tr}/P_{\text{total}}$  values, and  $P_r$  contents were obtained by difference.



**Fig. 3** Relationship between maize second leaf growth and the estimated phytochrome photoequilibrium established by the incident radiation. Seedlings pre-grown for 7 days in a white-light cabinet were transferred to the four light quality cabinets (see Fig. 1 legend). The length of the second leaf was measured from the coleoptilar node to the tip at day 7 ( $t_0$ ) and at the stated times later. *a*, Norflurazon-treated seedlings, leaf length measured at  $t_{48}$  and the increment expressed as a proportion of the length at  $t_0$ ; *b*, seedlings untreated with herbicide, leaf length measured at  $t_{24}$ ,  $t_{48}$  and  $t_{72}$ , each 24-h increment in length being plotted directly; *c*, as in *b* but with each 24-h increment plotted as a proportion of the leaf length at the beginning of that 24-h period (that is,  $t_i$ ); *d*, as in *b* but transferred to the treatments cabinet for 1 h only, followed by growth in complete darkness. The standard errors for all measurements were between 4% and 8% of the means.

previously observed in a range of other plants<sup>3–9</sup>, supporting recent claims that the herbicide treatment has little effect on phytochrome-mediated responses<sup>12–14,17</sup>.

To measure growth rate at daily intervals green (that is, not herbicide treated) seedlings were used. The absolute growth rate data in Fig. 3b show that the daily increments in leaf length ( $\Delta l$ )



were inversely related to the estimated phytochrome photoequilibrium in an approximately linear manner throughout the 3-day treatment period. Comparison with Fig. 2 shows clearly that although growth rate during the first 24 h was inversely related to  $P_{fr}$  amount (or concentration) at  $t_0$ , such correlations became progressively more difficult to make until, at  $t_{48}$  to  $t_{72}$ , growth rate appeared independent of  $P_{fr}$  amount. Figure 3d shows that the growth differences recorded in Fig. 3a–c are not merely effects of the light quality transitions at  $t_0$  and must be due to continuous modulation of growth rate by light quality throughout the  $t_0$ – $t_{72}$  period. These data show that growth rate is not a function of the  $P_{fr}$  amount or concentration. Nevertheless, during the whole 3-day treatment period, growth rate is clearly a function of photoequilibrium and, therefore, under phytochrome control.

These observations are not consistent with the simple ' $P_{fr}$  as active form' theory, nor can they be readily accommodated by the current complex models of phytochrome action<sup>16,18</sup> based on  $P_{fr}$  as the active form. To account for phytochrome action in light-grown plants, therefore, we must abandon the present doctrinaire adherence to the view that  $P_{fr}$  is the only active form of phytochrome.

I thank Mr Michael Jackson for technical assistance, Mr C. Kelsby and Mr J. Wall for the original discussions on which these experiments were based, and Sandoz AG for a gift of norflurazon.

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## Xenon as a solvent

P. M. Rentzepis & D. C. Douglass

Bell Laboratories, Murray Hill, New Jersey 07974, USA

The properties of pure xenon have been extensively studied and techniques have been developed for its use in, for example, the preparation of inert solid matrices, the filling of ionization chambers and as an anaesthetic in medical research. However, the use of liquid Xe as a solvent has been limited to a few small-molecule solutes such as hydrogen halides and formaldehyde, in a temperature range below and near the Xe melting point<sup>1–7</sup>. We now present experimental evidence in the form of UV, visible, IR and NMR spectra which show that liquid Xe can be used as a fluid solvent for several biological and organic molecules at temperatures from near room temperature to  $\sim -100^\circ\text{C}$ . Solid Xe solutions also provide a means of studying spectra of large or heat-sensitive molecules which cannot be investigated by traditional matrix isolation methods.

Xe is particularly useful among the rare gases because its liquid phase occurs in the fairly convenient conditions of  $\sim 16^\circ\text{C}$  and 58 atm so that solution studies can be carried out near room temperature. Its large polarizability relative to that of other rare gases means that Xe may be expected to have significant, but not chemically or structurally disruptive, interactions with solutes:

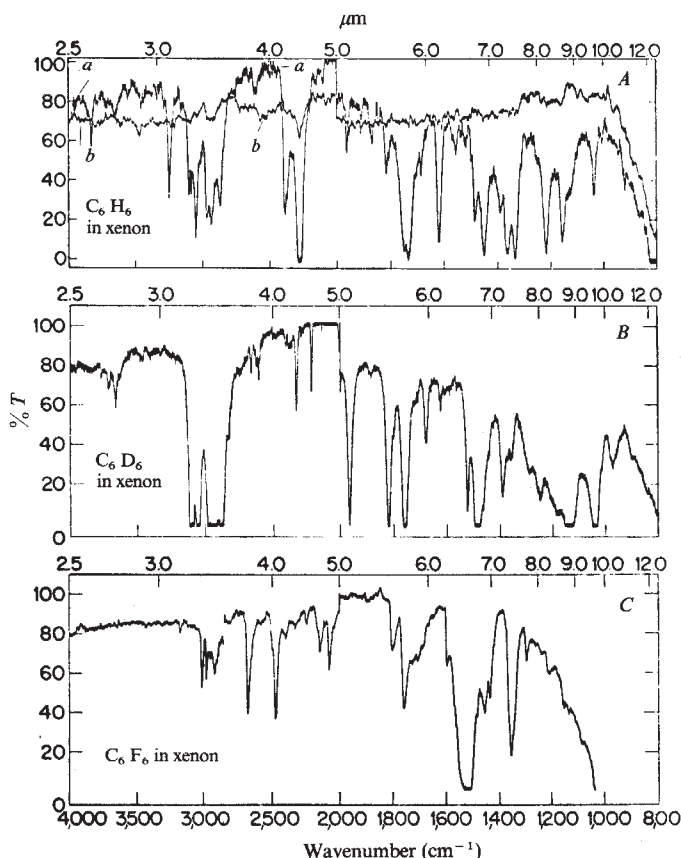
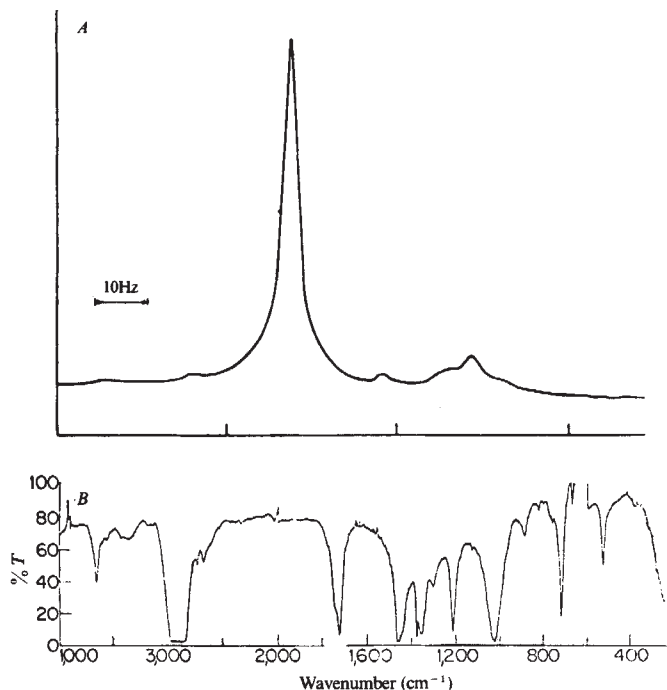


Fig. 1 IR spectra of benzene(s) dissolved in liquid xenon at  $\sim 14^\circ\text{C}$ . Recorded by a Perkin-Elmer IR spectrophotometer. A,  $\text{C}_6\text{H}_6$  dissolved in liquid Xe. B,  $\text{C}_6\text{D}_6$  dissolved in liquid Xe. The curve labelled *b* is a background spectrum and the curve labelled *a* is  $\text{C}_6\text{D}_6$  absorption. C,  $\text{C}_6\text{F}_6$  in liquid Xe.

Xe should approach the behaviour of an ideal inert solvent. Because of its optical transparency in most of the vacuum UV and all of the UV, visible and IR spectral regions, spectra may be recorded with Xe over a very wide spectral range without altering the character of the molecular environment. Moreover, change in spectral features arising from an environmental change, solvent fluorescence or spectral interference, is also avoided. When the solutions are cooled to low temperature and frozen, sharp bands similar to those observed for frozen matrices are often observed. The use of Xe solutions is therefore not restricted to vaporizable molecules, as is the case for low-temperature inert matrix media.

As Xe liquefies below  $16^\circ\text{C}$ , and can thus be solidified by lowering the temperature still further, solute spectra can be monitored continuously from the gas phase through the liquid state and into low-temperature solids. The same observation cell may be used over the entire range of conditions by modest pressure and easily implemented temperature changes. We believe this to be a novel system for studying gas, liquid and solid properties over a wide range of density and temperature. Our technique offers the possibility of carrying out many types of kinetic and absorption, emission, Raman and NMR spectroscopic studies.

The apparatus used for the Xe experiments consists of a high-pressure, low-temperature distillation system which allows purification and storage of Xe under high pressure. The optical cell is machined from stainless steel and usually contains four windows, a thermocouple and a transfer tube. The cell windows are 5/16 inches thick and 9/16 inches in diameter. They are sealed with viton or indium o-rings and held in place by stainless-steel flanges. The windows are KRS-5 or CsI for the IR region and quartz or sapphire for the UV and visible regions. The cell is attached to the end of a low-temperature Dewar for



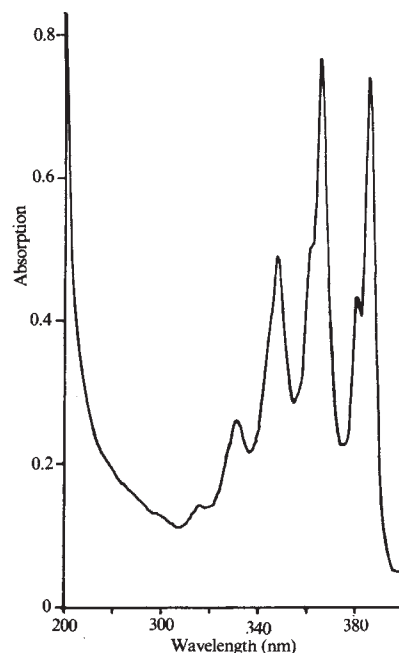
**Fig. 2** A, 90 MHz proton NMR spectrum of a ~15% solution of hexadecane in liquid Xe at 0°C. B, IR spectra of hexadecane dissolved in liquid Xe at ~14°C.

temperatures >77 K and to a cryotip for temperatures <77 K. The optical path length of the cells ranges over 2–8 mm.

Optical spectra are obtained in the following way. Solute is introduced into the bottom of the cell and the windows are sealed. A background spectrum is recorded before admission of Xe, the cell is pressurized with room temperature Xe to ~1,000 p.s.i. and a second background spectrum is then recorded. Next the cell is cooled 0–15 °C and allowed to fill with liquid Xe. Spectra of the solute are then recorded until absorption reaches a plateau. The Xe used in all our experiments may be returned from the cell through a purification column to a reservoir, and so may be used repeatedly.

Figure 1 shows the IR spectra of benzene, D<sub>6</sub>-benzene and perfluorobenzene in liquid Xe. The spectrum of benzene in Xe clearly differs from that of benzene alone. The large change in absorption in several regions of the spectrum indicates the presence of Xe–benzene interactions, perhaps analogous to those observed in gaseous rare gas/hydrogen halide mixtures<sup>8,9</sup> as well as in solid matrices<sup>10</sup>.

Solutions of benzene and toluene in Xe, at 0°C and equilibrium vapour pressure, show NMR dilution shifts of ~10%, which is nearly the same as observed for the corresponding carbon tetrachloride solutions, that is,  $\delta_1 - \delta_0 \approx 0.65 - 0.70$ . Hydrocarbons of modest size are readily soluble in Xe. For example, Fig. 2 shows the 90 MHz proton NMR and IR spectra of hexadecane in liquid Xe. The NMR spectrum corresponds to a 15% solution at 0°C and the IR spectrum to a less concentrated solution at 14°C. In the NMR experimental conditions used here, resolution is significantly compromised by the necessity of obtaining a spectrometer lock signal from a sample of



**Fig. 4** The UV spectrum of 9-bromoanthracene in Xe at 14°C.

D<sub>2</sub>O in an annular space surrounding the thick-walled 5-mm NMR tube. The sample tubes were generally sealed with a small KEL-F valve and viton o-rings.

Other molecules soluble in Xe include hydrocarbons such as hexane and dodecane, organic acids, methyl ethyl ketone, ethers and even polymeric viscous liquids such as polypropylene glycol (molecular weight ~4,000), the IR spectrum of which is shown in Fig. 3. Water is also soluble in Xe and several biological molecules are very slightly soluble (~10<sup>-6</sup> M).

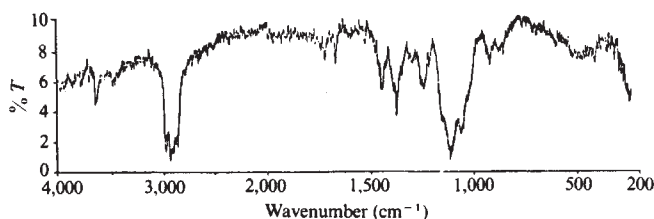
Even at low solubilities spectra are often sufficiently well resolved and intense to permit vibrational assignments and kinetic studies with visible and UV spectroscopy. For example, Fig. 4 shows the UV spectrum of 9-bromoanthracene in liquid Xe solution at ~14°C.

Clearly, a large number of molecules can be expected to be soluble in liquid Xe and thus their spectroscopic reactions and relaxation properties can be studied over wider ranges of conditions than is readily available with any other single medium. Experiments in which relaxation of excited molecules is measured from the isolated molecule to high density conditions will be reported elsewhere.

We thank Dr P. C. Milner for valuable discussions and Mr S. D. Rand for technical assistance.

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**Fig. 3** IR spectrum of polypropylene glycol dissolved in liquid Xe at 14°C.

## Voyager 1 at Saturn

The new results from Voyager 1, along with the description of the Voyager 2 mission by project director Dr Edward Stone, which appeared in *Nature* of 20 August are being reprinted in a glossy, 88-page booklet.

Copies can be ordered from Nature's offices in London, Basingstoke and New York at the following prices (including postage): UK £2.00 each; USA & Canada US\$4.00; Rest of World £2.50 (surface), £3.00 (air).



## MATTERS ARISING

## A chemical approach to orogenesis

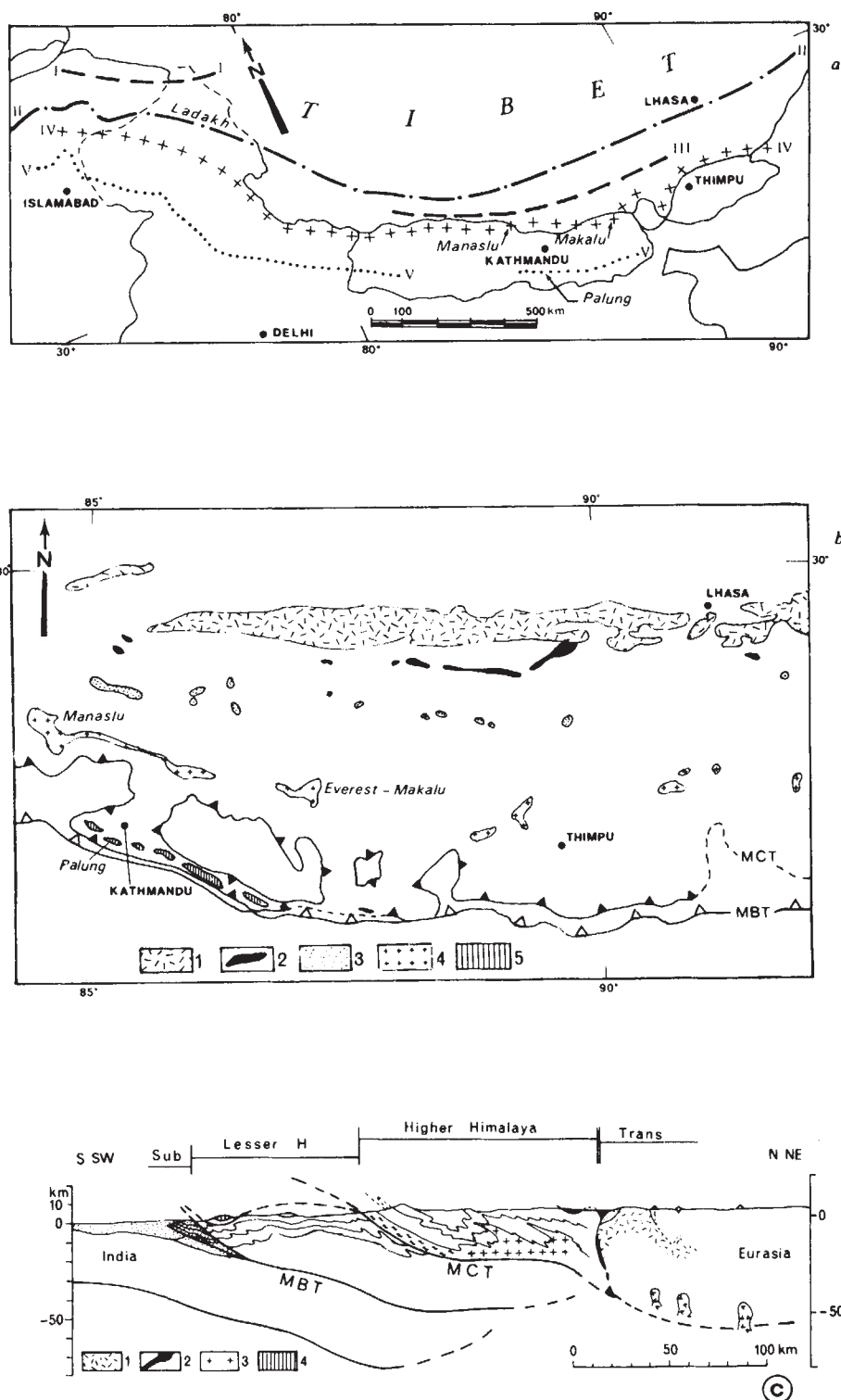
ALLÈGRE AND BEN OTHMAN<sup>1</sup> have used the Nd-Sr isotopic relationship to determine the genesis of granitoid rocks. They classify granitoid rocks younger than ~350 Myr into four different geodynamic environments: (1) island arcs (Japan); (2) continental subduction zones (Andes); (3) complex subduction areas (Southern California Batholith, Sierra Nevada) and (4) continental collision zones (Himalayan and European Hercynian granites).

Here I point out that there are problems with such a generalized classification. In particular their interpretation, at least of the 'continental collision situation' with which I am most familiar, is misleading in the use of literature and ignores the established geological framework of the Himalayan granitoids.

Figure 1 illustrates the principal granitoid belts of South Central Asia; each lineament represents a suite of granitoid rocks of characteristic petrology, geochemistry and age. Himalayan granitoids have been divided into two groups: the so-called 'tourmaline granites' of the Great or Higher Himalaya and the 'granites-granodiorites' of the Lesser Himalaya<sup>2</sup>. The Higher Himalayan granites are leucocratic, aluminium- and alkali-rich two-mica granites of probable post-Eocene and pre-Pliocene age<sup>3-6</sup>. The Lesser Himalayan aluminous granites have been shown<sup>7,8</sup> to form a series of disconnected plutons within a linear belt of Lower Palaeozoic age. As for the Transhimalayan—or Ladakh—batholith, it belongs to another mountain range and is really a complex association of plutonic rocks, possibly of Upper Cretaceous to Eocene age<sup>9</sup>.

The Ladakh batholith is stated<sup>1</sup> to "represent the granitoids associated with the major collision between India and Eurasia". In plate tectonic terms such a statement is misleading. A more likely interpretation is that the batholith corresponds to an island arc<sup>10</sup> and there is a general agreement that it represents the trace of the subduction of the oceanic floor of the Tethys during its closure. It should thus have been included in one of the three other proposed geodynamic environments.

Such confusion on a regional scale also persists on a more local level. The Makalu is associated to Kakani as the Makalu-Kakani granitoid with no explanation. One is a tourmaline-beryl-bearing pegmatite 6 km north of Kathmandu (Kakani), whilst the other is a Higher Himalayan granite (the Everest granite) 200 km away from Kakani. Similarly the



**Fig. 1** Localization of some granitoid belts close to the Indo-Eurasian boundary. *a*, The five main granitoid trends are: I, Karakorum; II, Transhimalaya; III, North Himalaya; IV, Higher Himalaya; V, Lesser Himalaya (adapted from ref. 15). *b*, Granitoids belts of the eastern central part of the Himalaya and Transhimalaya, with the localization of the main Himalayan plutons. 1, Transhimalayan batholith; 2, ophiolites of the Tsangpo suture; 3, North Himalayan alignment; 4, Higher Himalayan leucogranites; 5, Lesser Himalayan granites; MCT, main central thrust; MBT, main boundary thrust. *c*, Cross-section showing the localization of the three main belts of granitoid<sup>5</sup>: 1, the Transhimalaya batholith, north of the suture; 2, ophiolites of the Tsangpo suture; 3, Higher Himalayan leucogranites; 4, Lesser Himalayan granites.

Manaslu, Makalu and Palung plutons are grouped together even though these granites come from two different belts with different petrologies and chemistries, and differ in age by nearly 500 Myr (refs 7, 8).

A whole rock Rb-Sr isotopic age determination has been published<sup>11</sup> on samples of the Manaslu leucogranite that I collected in 1974. The age, given as 29 Myr in the text and as 28 Myr in the table, was first questioned by Vidal<sup>12</sup>. Subsequent studies by Vidal on the same samples and also new samples collected in 1975 have confirmed the complete lack of an isochron: the data only give a scatter diagram<sup>13,14</sup> of which Allègre at least was aware.

Furthermore the initial Sr ratios are said to be "around 0.780", a value obtained as some average between the value of 0.7408 for the Manaslu granite (Higher Himalaya, probably Miocene) and 0.8000 for one sample of the Palung granite (Lesser Himalaya, Lower Palaeozoic). Can such averaging have any geological or geochemical significance?

Let us now consider the Himalayan data in Table 1 of Allègre and Ben Othman<sup>1</sup>. For the Ladakh batholith, on samples collected by a group including myself, HB 74, a diorite, is a tonalite, and HB 68, a granodiorite, is actually a quartz-monzodiorite. In addition to this confusion, the values for  $\epsilon_{\text{Nd}}$  in the two columns of their Table 1 are neither the same nor correct when recalculated using the data given in the other columns. The sources or the reliability of the 'real age' values listed are difficult to establish: 28 Myr is from the no longer valid isochron for the Manaslu; 26 Myr for the Makalu has never been published; and 51 Myr for Ladakh deserves an explanation given that many others have failed to obtain an age. There are also inconsistencies with the  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios given for Ladakh in Table 1. The ratios were never published by Hamet and Allègre<sup>11</sup>; in fact the two samples were only collected during the summer of 1976.

Finally many of the references given concerning the Himalayas are misleading, inadequate, or ill referenced.

Although I have only looked closely at the Himalayan section, the above comments must question their conclusions. Despite this, the Allègre and Ben Othman article presents an important group of Nd isotopic results. But surely progress in geochemistry cannot be made by ignoring the geological relationships.

PATRICK LE FORT

Centre de Recherches Péetrographiques  
et Géochimiques,  
BP No. 20,  
54501 Vandœuvre lès Nancy, France

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ALLÈGRE AND D. BEN OTHMAN  
REPLY—LE FORT'S does not really challenge the main point of our interpretation, namely the participation of recycled crust in the formation of the Himalayas granitoids. His detailed points do not challenge the increase of such recycling with time.

We stated that the granitoids seem to be slightly different and not well dated either. Space constraints prevented any details; however, these would not have affected our conclusion. As the ages range from Trias to Miocene, the  $\epsilon_{\text{Nd}}$  initially determined is independent of age. The quoted values of the  $^{87}\text{Sr}/^{86}\text{Sr}$  initial ratio ( $\sim 0.780$ ) are indeed not very precise as we stated but, despite the spread in the data our basic conclusion is correct. In any case, they are very different from mantle values around 0.702 to 0.705. A detailed age discussion would not be warranted at this stage. We believe that the Ladakh granitoids' origin is only a minor problem here. We used the expression "associated with" because we did not want to enter into the debate about their origin. Le Fort interprets it as an island arc but others differ especially with respect to the age of collision which seems to be Cretaceous (F. Proust and M. Colchen, personal communication), whereas Ladakh seems to be a complex body with different plutons which give a variable age from 45 Myr (ref. 1). Again, we did not want to contribute to this controversy as our objective is very general and we wanted to consider the tectonic situation at large. Whatever the detailed geodynamical process presumably Ladakh would still be "associated" with the Asia-India collision.

Andrieux *et al.*<sup>2</sup> have already presented a tectonic interpretation of the general area of Makalu; thus a discussion of Makalu would be irrelevant to our paper. We are more interested in their place in general tectonics. For example Le Fort previously interpreted<sup>3</sup> this kind of granitoids as linked with the so called "reverse metamorphism" of the MCT. We know now that this "reverse metamorphism" is

a theoretical model which is no longer consistent with recent field data<sup>4</sup>.

Note that we have tried to avoid such genetic debates and that we only consider the large tectonic relationships from this point of view. Mattauer<sup>5</sup> was the first to interpret the Hercynian chain in western Europe in terms of collision tectonics. Granitoids are part of an orogenic segment. As we clearly stated, we use this type of general argument to put the Hercynian granites in such categories.

Le Fort mentioned inappropriate referencing. We agree that we could have provided a much more complete set of references; however, given the scope of our paper we only had space to refer to a few important papers. But we note that as three of the key references given by Le Fort are 'in the press' they cannot be quoted by us.

We thank Le Fort for pointing out that there are obvious dangers in developing a general model such as ours on the basis of a small number of samples. And, of course, we agree that, ultimately, close attention has to be given to the detailed regional geology. Nonetheless, we feel that our more global approach has an important role to play. The required corrections affect neither the conclusion of our paper nor the general line of reasoning.

C. J. ALLÈGRE  
D. BEN OTHMAN

Laboratoire de Géochimie  
et Cosmochimie,  
Universités de Paris  
6 et 7, 4 Place Jussieu,  
75230 Paris Cédex 05, France

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## Corrigendum

In the letter 'Ordering of aluminium and silicon in synthetic faujasites' by S. Ramdas, J. M. Thomas, J. Klinowski, C. A. Fyfe and J. S. Hartman (*Nature* **292**, 228-230; 1981), the sixth paragraph should begin 'Closer examination of Fig. 2 reveals that para occupancy of type II rings begins at Si/Al = 1.40 and not at Si/Al = 1.67 as postulated by Dempsey *et al.*<sup>2</sup>. We suggest . . .

## Erratum

In the letter 'The phosphatidylinositol cycle and the regulation of arachidonic acid production' by E. G. Lapetina, M. M. Billah and P. Cuatrecasas (*Nature* **292**, 367-369; 1981), the last sentence of the first paragraph should read 'The correlation in platelets of a phosphatidylinositol response and the deacylation of the resultant phosphatidate by a specific phospholipase A<sub>2</sub> might suggest that these phenomena are applicable to activations in other cell systems'.



## BOOK REVIEWS

## French science under a benign bureaucracy

Robert Fox

SINCE the 1660s, when Colbert first perceived that the act of patronizing the nation's *savants* might add to the lustre of the Sun King, French science has depended on the support of government to a degree unmatched elsewhere in Europe. The state of dependence has on occasions shown its darker side: in the 1850s, for example, Napoleon III's Ministry of Public Instruction was a byword for parsimony and for incomprehension of the true needs of scientific research. But, in general, the story has been otherwise, with scientists (in search of funds and public authority) and statesmen (in search of national prestige and technical expertise) finding a community of interest unknown in other realms of intellectual life.

The community of interest was never more evident than in the period treated in Professor Gillispie's learned and absorbing book. That, at least, is the implication of his meticulously detailed account of the main institutions and personalities of French science in the 15 years that separated the beginning of Turgot's ministry in 1774 from the Revolution. By then, the Enlightenment, as we normally define it, was virtually over; yet enlightened spirits survived in the administration, working not at criticism and abstract prescription (in the manner of the Encyclopaedists) but at the practical business of rationalization and reform. In their task, modern-minded political leaders from Turgot to Breteuil looked to men of science to advise on the production of gunpowder and munitions, to improve public health and agriculture, to man the state-owned manufacturing enterprises at Sèvres and the Gobelins, and to serve as (and in turn to train) engineers in the various state corps, both civil and military. In the process, they not only brought together the worlds of science and polity but also, and even more importantly for Gillispie, caused an unprecedented expansion of the institutional provision for scientific work and education.

Gillispie's interpretation rests on a belief that the state's involvement was almost invariably benign. He shows how scientists and engineers of the stature of Lavoisier, Berthollet, Vicq d'Azyr, Monge and Coulomb, all of whom benefited from the new possibilities of career-making, were glad to assume the mantle of public servants, seemingly untroubled by any thought that such roles might conflict with their quest for a scientific reputation or damagingly reduce their freedom. The analysis of *Science and Polity* makes good sense of this compliance. For the accolade of official recognition and the opportunity

*Science and Polity in France at the End of the Old Regime.* By Charles Coulston Gillispie. Pp.601. ISBN 0-691-08233-2. (Princeton University Press: 1981). \$52.50, £28.20.

of appearing as the benefactors of society gave welcome substance to the scientists' claims to professional status. It is no wonder, therefore, that the state was regarded as the benevolent partner of the scientific community, while the enemies of science were identified elsewhere, in the world of the charlatans on the shady fringes of respectability. The very fact that certain pretenders to scientific competence were instantly recognized as threats is the surest touchstone of an incipient professional self-consciousness which united the Academy of Science in its opposition to Mesmer's notions of animal magnetism and in its frosty disregard of the physicist and future regicide, Marat. At the time, there could have been no conception of the age of the professional academic that was to dawn in the nineteenth century. But the possession of esoteric natural knowledge was already seen as a bond which distinguished such diverse groups as doctors, engineers, astronomers and industrial chemists from the rest of society, not least from the cultivated men of letters. Increasingly, and quite irrevocably by the time of the Revolution, it set the trained technical expert apart from, and in his own view above, those encyclopaedic generalists who had embodied enlightenment only 30 years before.

Readers of this book who know Professor Gillispie's earlier writings will recognize in it some of his favoured themes. His conviction of the nobility of the scientific enterprise is undimmed, though his handling of the quacks, Mesmer and Marat, is restrained and sensitive; his preference for doers rather than the layers of plans and the writers of manifestos is evident in his admiration for Turgot and (presumably) in the meagre space allotted to the wordy and ineffectual *idéologues*. Above all, there is scholarship as thorough as ever, and a knowledge of French ways and a love of France without which no Anglo-Saxon could ever have embarked upon such a formidable task. As a comprehensive work of reference on the official face of French science in the 1770s and 1780s, it is hard to imagine that *Science and Polity* will ever have its equal. There is still, of course, ample room for studies of the unofficial, voluntarist tradition, perhaps with a focus on the provincial academies or on private scholars and the teachers in the provincial *colleges* and

universities. But the real meat of French science in the later eighteenth century is undoubtedly in these 600 closely printed pages.

Professor Gillispie's friends and classes of grateful pupils at Princeton have had occasion to know that this book has been long in the writing — inevitably so, for in both conception and execution it is a work on the grand scale. I can pay it no greater compliment than to say that the wait has been well worth while. □

*Robert Fox is Reader in the History of Science at the University of Lancaster and President of the British Society for the History of Science. His most recent book, co-edited with George Weisz, is The Organization of Science and Technology in France, 1808–1914 (Cambridge University Press, 1980).*

## Hoyle on ice

G. de Q. Robin

*Ice: How the Next Ice Age Will Come and How We Can Prevent It.* By Fred Hoyle. Pp.191. ISBN 0-09-145320-8. (Hutchinson: 1981.) £7.95.

IN my research-student days, a colleague with literary ambitions threw his draft thesis on nuclear physics out of the window and settled down to write real fiction instead, although without much success. Fred Hoyle, in contrast, has succeeded in producing outstanding work in both astrophysics and science fiction.

The reader of *Ice* may well ask whether this is a serious scientific study or a piece of science fiction. Although ostensibly written as the former, the answer is not entirely clear. Basic scientific concepts are described to the layman with considerable skill, but more emphasis appears to be given to the dramatic idea than to a balanced scientific assessment.

Hoyle reviews a wide range of evidence on ice ages, taken from biological as well as physical sciences. The disappearance of biological species over short periods and geological evidence convinced him that climatic changes such as the onset and ending of ice ages take place suddenly. This leads to the hypothesis that ice ages are started by the impact of vast stony meteorites (of the order of 1 km in diameter) and ended by impact of similar iron meteorites. The dust from the first impact shrouds the Earth so effectively for a decade that the surface is cooled rapidly, leading to the formation of extensive ice cover over land and chilling of the warm

upper layers of the ocean. The ice sheet survives due to colder conditions maintaining a protective screen of ice crystals (diamond dust) in the atmosphere. A further necessary condition is the presence of a large continent at a high latitude.

The author draws on many scientific papers and books to make his case. His sections on plate tectonics, on the Earth as a heat engine and on the probability of asteroid impacts give useful summaries, as do the technical notes on radiocarbon dating, on the Earth's magnetic polarity and on oxygen isotope analysis.

However to make the case of his "instant" ice age, the author has to reject too many well-established concepts to convince a scientific mind of the validity of his model. He refers to a "mistaken belief that ice ages were caused by an enormous expansion of mountain glaciers". He states that "Ice can, of course, flow any distance, as long as it does so on a downward slope, but across the North Sea we are not concerned with a downward slope". Because he considers ice cannot flow over flat surfaces, he rejects the idea that Scandinavian erratics could have been carried to England by ice sheet motion and returns to the ice rafting concepts of last century. On inland regions, he suggests that erratics skate over smooth ice sheets, even being blown uphill. It is not quite clear if he is serious, as his introduction refers to this by saying, "If my attempt to find such a different process does not commend itself, I am happy to leave it to the reader to find a better one!". His proposal to prevent the next ice age by pumping up deep ocean water for a millenium or more must be seen as science fiction at this time.

The search for evidence of meteorite impacts and their effect on the Earth is a challenging problem that was recently discussed by Kyte *et al.* (*Nature* 292, 417). They found traces of meteoric debris of 2.3 million years of age in a deep core from the Antarctic Ocean, but this was not associated with any biological extinctions or environmental changes. However with a minimum diameter estimated at 20 m, it may not have been large enough to test the ice age hypothesis. The increasing evidence in favour of solar orbital hypotheses as a cause of ice ages is ruled out by Hoyle because the total input of heat to the Earth varies little, and he considers latitudinal variation of heat input insufficient to cause ice sheet growth.

It seems surprising that Sir Fred Hoyle, who has developed the idea of continuous creation in the Universe, should go for a "big bang" theory of climate at this time. This is a pity as, with some restraint, more consideration of recent evidence such as that from ice cores and some limited changes of argument, the book could have provided an excellent layman's guide to problems of climatic change and ice ages.

*G. de Q. Robin is the Director of the Scott Polar Research Institute, University of Cambridge.*

## A voyage to the ancient oceans

James P. Kennett

*Paleoceanography.* By T.J.M. Schopf. Pp.341. ISBN 0-674-65215-0. (Harvard University Press: 1980.) \$32.50, £17.50.

UNTIL now, no book has attempted to bring together the various aspects of the burgeoning field of palaeoceanography, the newest of earth science disciplines and one almost undreamed of only two decades ago. Thomas Schopf's *Paleoceanography* thus provides an important compilation of material about the ancient oceans. His is a holistic view — the recognition of the close interactions between the major components of the Earth's environment. The breadth of inquiry is large, as required for any synthesis of data and theories on the ancient ocean's environmental conditions; for instance, the reader will move from discussions about oxygen and carbon isotopes, to taxonomic diversity, to boron as a palaeosalinity indicator.

Schopf asks important questions about the evolution of the global ocean related to its origin, its volume, its chemistry and its life. For each of seven major parameters analysed, such as temperature and biology, he includes summaries of modern conditions, methods of study and history of change. We learn, for instance, that global biological productivity has not varied by more than a factor of two during the last 3.3 billion years, even though taxonomic groups have undergone considerable change.

An overview is attempted of the evolution of the oceans during the entire history of the Earth, but the size of this topic has naturally limited the scope of the book. Conspicuously absent, for the most part, are the recent findings on the deep ocean basins and pelagic realm which have resulted from the study of deep-sea sediments. The deep-sea drilling project is barely mentioned. Instead Schopf has concentrated his efforts upon the shallow-water marine sedimentary record and inevitably upon the pre-Mesozoic ocean. He is at his best when describing the Precambrian ocean. Those more interested in the late Phanerozoic record will need to look hard for information; nevertheless, Schopf usually attempts to work from first principles, and thus the student interested in any period will be enlightened.

Although each of the chapters is well organized, the overall focus of the volume is somewhat elusive. This partly results from the lack of a final synopsis which could have usefully been included to integrate the numerous concluding statements from each of the chapters. A coherent evolutionary picture of the oceans does not emerge easily from this volume.

Despite its limitations, this is a most informative and stimulating book which should be read by all students of the ocean; after all the oceans are the product of their

history. The majority of palaeoceanographers — those working in the Cenozoic and late Mesozoic — will find the ocean as viewed by Schopf to be largely unfamiliar, but it should strengthen their understanding of more recent oceanographic evolution. The book has broad appeal and I would also recommend it as a text in senior-level courses dealing with palaeoceanography and marine palaeoenvironments, not so much for its comprehensiveness, but because it will promote awareness of the more unfamiliar ancient ocean. □

*James P. Kennett is a Professor of Oceanography at the University of Rhode Island, Kingston.*

## Malaria: still cause for concern

R.J.M. Wilson

*Malaria.* Edited by Julius P. Kreier. Vol.1 *Epidemiology, Chemotherapy, Morphology, and Metabolism*, pp.416, ISBN 0-12-426106-9; Vol.2 *Pathology, Vector Studies and Culture*, pp.328, ISBN 0-12-426102-7; Vol.3 *Immunology and Immunization*, pp.345, ISBN 0-12-426103-5. (Academic: 1980.) Vol.1. \$49, £32.40; Vol.2. \$38.50, £25.50; Vol.3. \$39.50, £26.20.

MALARIA remains the most important parasitic disease of human beings in terms of mortality and morbidity. In the early 1950s, malaria eradication was attempted in many countries and as a result the disease receded from the temperate zone where its epidemiology was unstable. There has been, however, a major resurgence of endemic malaria in Central America and Southern Asia due to the emergence of vector mosquitoes resistant to insecticides and parasites resistant to chemotherapeutic drugs, and to a reduction of malaria control measures as financial resources have been withdrawn. Today, the malarious regions of the world still encompass some 1,600 million people. Even in peripheral areas such as the UK, cases of malaria arising from business and tourist travel increased from 134 in 1970 to 2,503 in 1979. Clearly, the slackening of malaria control measures has had serious consequences, and in this context it is ironic to note that the UK government has recently withdrawn its support to the WHO's well-publicized Six Diseases Programme with its strong emphasis on malaria.

In this volatile situation, a new broadly-



based treatise on malaria would be most useful. Unfortunately this trilogy is not what we might have hoped for. An opportunity to update the invaluable works of Boyd (*Malaria*; Saunders, 1949) and Garnham (*Malaria Parasites and Other Haemosporidia*; Blackwell Scientific, 1966) has thus been lost.

The topics covered by the three volumes now offered include epidemiology (glaringly misspelt on the title page), chemotherapy, morphology and metabolism (Vol.1), pathology, vectors and parasite cultivation (Vol.2) and immunology (Vol.3). The underlying fault, I feel, is the irritatingly disorganized substructure of many of the chapters in relation to the book as a whole. For example, in Vol.1 Wernsdorfer's opening chapter on "The Importance of Malaria in the World" commences not with epidemiology, but with a detailed review of parasite taxonomy with no fewer than 13 pages of taxonomic tables. Before getting round to his excellent summary of the epidemiology of malaria — a subject that deserved an expanded chapter on its own — Wernsdorfer also describes the life cycle of the parasite, a topic that would have been better dealt with by re-siting the later chapter on "The Morphology of Plasmodia".

In the next contribution, "Malaria in its Various Vertebrate Hosts", Garnham manages, to his credit, to keep the section on reptilian malarias well within bounds (three sentences), and writes a storming account that romps through all aspects of the malaria parasite's interaction with the host and how disease results. Again, his interesting descriptions of pathology would have been more appropriate in the chapter devoted to this aspect in Vol.2, to which Garnham has to refer. A further example concerns the role of blood groups, receptors and haemoglobinopathies. These receive passing mention several times in each volume, without the critical assessment that a specialized section or chapter might have given. In the remaining chapters of Vol.1, Peters is a little short on molecular aspects of drug action but otherwise thorough, whilst Homewood and Neame continue to debunk the hard-won but sometimes suspect biochemical literature.

Volume 2 opens with two uninspiring chapters on pathology (to find mention of algid malaria or pyrexia one has to go back to Vol.1 — even here algid malaria is not indexed). I think that for medical readers these chapters will be particularly lacking in breadth of interest. In contrast, the following sections on mosquitoes and the parasite's developmental cycle therein, are practical and informative for the non-specialist. The challenge of cultivating the invertebrate stages of plasmodia is well expressed. Trager reviews various cultivation methods for erythrocytic and exoerythrocytic stages of malaria parasites but leaves unsaid the problems of suspension culture. Much emphasis is

placed on the use of RPMI 1640 but little on the importance of the gas phase. An interesting section summarizes his pioneering work on the extracellular growth of erythrocytic parasites.

It was annoying to find chapters even in Vol.3 still opening with statements such as "the malaria parasite develops in two hosts. . .", or "the malaria parasite is a complex eukaryotic organism. . .". In addition Chapters 1 and 3 of Vol.3 are marred by many typographical errors and spelling mistakes: is it "pellicular complex" (Aikawa, Vol.1) or "pellicle complexes" (Hamburger and Kreier, Vol.3), and what about "Egitt", "fc receptors" and "sequellae"? Grammatical howlers have crept through too — "plasmodia cause the red cell to enlarge by inhibition of water, thereby decreasing its density. . .".

Despite these quibbles, Vol.3 contains an excellent account of some real progress made by immunization against sporozoites, as well as an authoritative review of gamete immunization. Immunity to the exoerythrocytic stage is evaluated and the prospect of an important debate is glimpsed in Siddiqui's chapter on immunization against asexual blood forms of the parasite — "The aim of human malaria vaccination at the present time is not to obtain sterile immunity . . . but to modify the clinical course of the disease". This discussion is taken up in brief appendices where further voices are heard (with the seemingly inescapable repetition of literature sources). But the inclusion of general statements by funding organizations at the end of the book seems inappropriate — perhaps they felt neglected.

These volumes, then, contain a string of workmanlike reviews in typical Academic Press style: solid and informative but lacking the sharpness of an overall synthesis that might inspire the newcomer or revitalize the "jaundiced" specialist. □

R.J.M. Wilson is at the National Institute for Medical Research, Mill Hill, London.

## Ions in water

Martyn C.R. Symons

*Ionic Hydration in Chemistry and Biophysics. Studies in Physical and Theoretical Chemistry, 12.* By B.E. Conway. Pp.774. ISBN 0-444-41947-0. (Elsevier Scientific: 1981.) \$131.75, Dfl.270.

DESPITE its grey, forbidding outlook and its formidable bulk this book is well worth reading, and will undoubtedly become an important reference work for those concerned with ions in water. It covers a far broader field than most of its predecessors except those that are multi-authored. Brian

## A MAJOR PUBLISHING EVENT PARTICLE PHYSICS AND INTRODUCTION TO FIELD THEORY

By T.D. Lee

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Just published by Harwood, this original new work forms the initial volume of the series *Contemporary Concepts in Physics*. In nearly 900 pages Professor Lee offers a unified presentation of particle physics, starting with a self-contained discussion of quantum field theory and going on to the symmetry and interaction of particles. *PARTICLE PHYSICS* expresses the author's personal approach to the subject and contains many derivations and formulas which have not yet appeared in the literature.

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Tsung-Dao Lee was awarded the Nobel Prize in Physics in 1957. His major areas of research include particle physics, statistical mechanics and field theory.

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Conway has been an authority on the subject for many years and he is to be congratulated on completing this mammoth task so satisfactorily. I am full of admiration.

Inevitably, the work is not without flaws, some trivial, some rather more serious. It should be said, however, that even in sum these do not detract significantly from the importance of the book to those in this area of research.

The main thrust is towards the measurement and interpretation of thermodynamic and electrochemical properties of aqueous solutions of ions. This is both lucid and thorough. Less thorough and less critical attention is given to spectroscopic studies, and there is almost no mention of the powerful tools of X-ray and neutron diffraction. There is a chapter on non-aqueous solvents, but I do not feel that the results for such solvents are used to shed light on the aqueous solution problems. In addition, the ordering of topics seems to me to be somewhat arbitrary: thus, for example, the key topic of solvation number is not discussed seriously until Chapter 29. This chapter nicely illustrates some of the historical quirks of the subject, but I would have been happier had the firm information that is now available regarding solvation numbers been used more incisively.

Sections of interest to the biophysicist (and surely to biochemists, physiologists

etc.) include discussions on interfaces, transport through membranes and hydration of polyelectrolytes.

Chapters concerned with the application of physical techniques, for example NMR spectroscopy, are each preceded by a (necessarily) brief introduction. I feel that some of these introductions fall between two stools, being too curt for the uninitiated, but unnecessary for others. Since such topics as NMR spectroscopy have been routinely taught to undergraduates for many years, I think that a book at this advanced level could have assumed a basic knowledge of such techniques.

Having studied ionic solvation by ultra-violet spectroscopy for the past 27 years, I was sad to find no mention of the use of this technique, especially since infrared and Raman spectroscopic studies are given fair coverage. I also think that the book would have gained by a consideration of solvated electrons. These interesting entities have

been studied in great detail and many of the results are of significance to the problem of anionic solvation. However, it is easy to be critical, impossible to be comprehensive and certainly impossible to please everyone.

Finally, a small complaint about references. I realize that it is convenient to produce a list of references for each chapter. However, it would be far more convenient to the reader if these lists were collected together at the end of the book.

I will certainly recommend this work to all interested in ionic solvation: they can wander through this forest of information and make it their own task to "see the wood for the trees". For the more general reader, absence of such an endeavour on the author's part may make the book somewhat less satisfactory. □

Martyn Symons is Professor of Chemistry at the University of Leicester.

## Hesse on post-revolutionary reconstructions

R.G.A. Dolby

*Revolutions and Reconstructions in the Philosophy of Science.* By Mary Hesse. Pp. 271. ISBN 0-85527-268-6/0-253-33-381-4. (Harvester Press/Indiana University Press: 1980.) £20, \$22.50.

MARY Hesse, Professor of Philosophy of Science at the University of Cambridge, is one of the leading British philosophers of science. The present book, her fifth, collects together a selection of ten of her papers which have appeared since 1965. A specially written introduction has been added.

The book is concerned with the new empiricist philosophy of science which has emerged over the past few decades. Professor Hesse fully identifies with it, vigorously defending, for example, the theory-ladenness of all observation, and arguing that the importance of metaphor in science necessitates revision of the deductive model of scientific explanation. The primary purpose of the book is to help consolidate the new philosophy of science, by mapping out more clearly its central concerns and through careful discussion sorting the sensible from the silly.

The book begins with a discussion of recent historiography of science, seeking to explain some of its peculiarities in terms of reactions to its past philosophical fashions. The second paper is the only one which has been significantly changed for the present volume; it is a study of "the strong thesis of sociology of science". This is the claim that true belief and rationality (in science) are as much to be explained by social causation as are error and non-rationality. The discussion tends to the conclusion that this apparently provocative claim is much

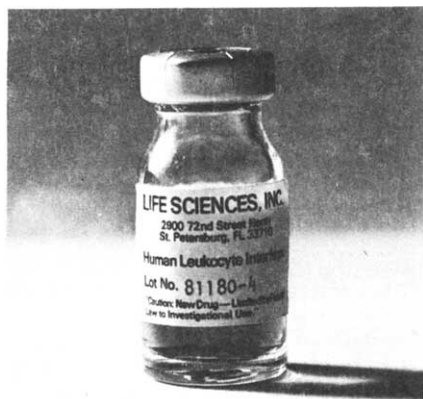
weaker than it at first appears. In the second section of the book, Professor Hesse returns to some of the themes of her earlier writings to do with the relation of observation and theory, the function of metaphor and the nature of theory change. In addition, she discusses what changes the new developments in philosophy of science imply for such central concepts as objectivity and truth. The third section develops these issues by focusing upon hermeneutical philosophies of the social sciences, especially in the work of Habermas. The paper in the final section looks at the implications for theology of such ideas as the consensus theory of truth which were discussed in the earlier sections.

A book such as this, which reprints learned articles, is primarily directed to a limited intellectual community centred upon philosophy of science. Much of it would be difficult reading for a more general audience. However, Professor Hesse is at her best in her lucid representation and examination of fashionable developments in related areas, from which she extracts the claims and arguments which have most interest to the new philosophy of science. In addition to recent history and sociology of science, for example, she discusses such figures as Habermas, Althusser and Monod.

This collection of papers is to be valued for its consolidation of the new philosophy of science rather than for breaking new ground, and will be a valuable resource for all who are interested in the field. □

R.G.A. Dolby is Senior Lecturer in the Unit for the History, Philosophy and Social Relations of Science, University of Kent at Canterbury.

  
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